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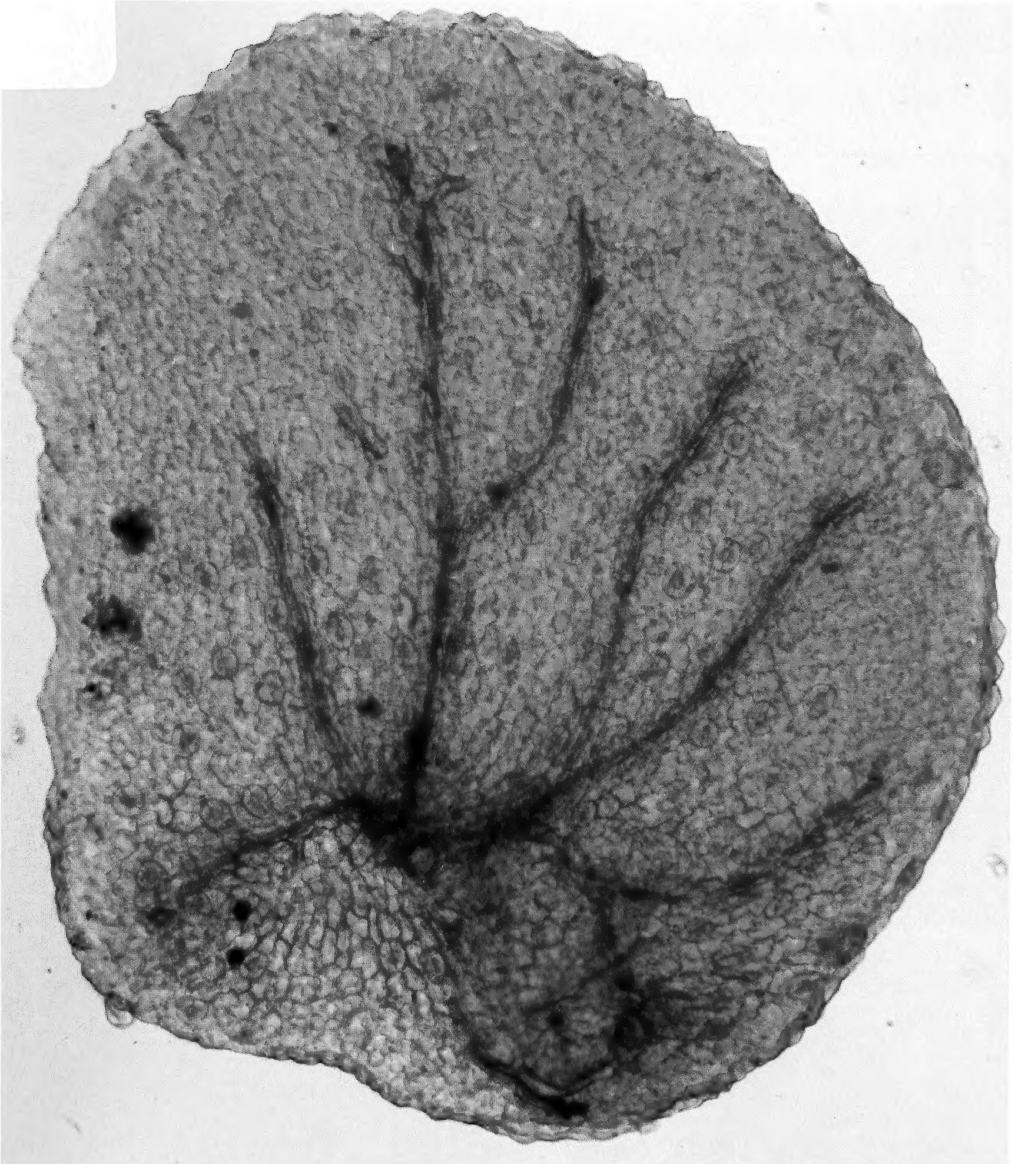
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Ultrastructural Studies of Oogenesis in the Fern *Diplazium glaucum*

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ABSTRACT.—The present investigation examines the oogenesis of the gleichenioid fern, *Diplazium glaucum* (Thunb. ex Houtt.) Nakai using transmission electron microscopy. The results demonstrate that the oogenesis of *D. glaucum* is similar to the core leptosporangiate ferns. The egg cell of *D. glaucum* was progressively isolated from the adjacent cells by forming a separation cavity and an egg envelope during maturation. However, during this process, a pore region consistently connected the egg and the ventral canal cell. Ultimately, a fertilization pore formed at the pore region when the egg matured. The nucleus produced conspicuous evaginations during later stages of egg development. The cytological features during oogenesis in *D. glaucum* more closely resembled the core leptosporangiate ferns rather than the basal leptosporangiate ferns (*Lygodium* and *Osmunda*), which possess no egg envelope or fertilization pore. The results provide new characteristics for interpreting the evolutionary history of the gleichenioid ferns.

KEY WORDS.—gleichenioid fern; egg envelope; fertilization pore

Observations of oogenesis among fern species representing the core leptosporangiate ferns including *Pteridium aquilinum* (Bell and Mühlethaler 1962a, b; Bell and Duckett, 1976; Cao, Dai, and Wang, 2012b), *Histiopteris incisa* (Bell, 1980), *Athyrium filix-femina* (Fasciati *et al.*, 1994), *Dryopteris crassirhizoma* (Bao *et al.*, 2005), *Ceratopteris richardii* (Lopez-Smith and Renzaglia, 2008), *Ceratopteris thalictroides* (Cao, Yang, and Wang, 2009; Cao, Wang, and Bao, 2010a), *Adiantum flabellulatum* (Cao *et al.*, 2010b), *Plagiogyria euphlebia* (Cao, Dai, and Wang, 2011), *Coniogramme emeiensis* (Wang *et al.* 2012a), *Cibotium barometz* (Wang *et al.* 2012b), *Anisocampium sheareri* (Yang *et al.* 2013), and *Phymatosorus hainanensis* (Zou *et al.* 2014) demonstrate that all mature eggs of these species possess an egg envelope consisting of a reticular or multilayered structure. In addition, all of these species possess a fertilization pore, a structure that limits penetration of the sperm (Cao, Yang, and Wang, 2009; Cao, Wang, and Bao, 2010a; Cao *et al.*, 2010b; Cao, Dai, and Wang, 2011; Cao, Dai, and Wang, 2012b; Wang *et al.*, 2012a, b; Yang *et al.*, 2013, Zou *et al.*, 2014). The structure and formation of the fertilization pore in the ferns had been described in *Ceratopteris* and *Pteridium* (Cao, Wang, and Bao, 2010a; Cao, Dai, and Wang, 2012b).

The basal groups of the leptosporangiate ferns include osmundaceous ferns, filmy ferns, gleichenioid ferns, and schizaeoid ferns (PPG I, 2016; Pryer *et al.*,

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2004; Smith *et al.*, 2006). Among these, *Osmunda*, possesses no egg envelope or fertilization pore in the mature egg (Cao, Dai, and Wang, 2012a). *Lygodium japonicum*, representing the schizaeoid ferns, only forms a pore region between the egg and the ventral canal cell during oogenesis, with no egg envelope or fertilization pore (Cao *et al.*, 2017).

Gleichenioid ferns lie between osmundaceous ferns and schizaeoid ferns in the fern phylogeny (PPG I, 2016; Smith *et al.*, 2006). However, the cytological features of the oogenesis of gleichenioid ferns have not been investigated. Whether the cytological features of the oogenesis of gleichenioid ferns reflects their phylogenetic status remains obscure. In the present investigation, oogenesis of the fern *Diplopterygium glaucum* is observed in detail, and the results provide some new evidence for understanding the phylogenetic position of the gleichenioid ferns.

MATERIALS AND METHODS

Spores of *Diplopterygium glaucum* (Thunb. ex Houtt.) Nakai (before revision called: *Hicriopteris glauca* (Thunb.) Ching) were collected from plants in Ningbo city, Zhejiang Province, China. The spores were surface sterilized with 5% sodium hypochlorite solution for 3 min. After rinsing three times with sterile distilled water, the spores were sown on a modified Knop's solution (0.8g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 0.2g KH_2PO_4 ; 0.2g KNO_3 ; 0.2g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, dissolved in 1 liter distilled water), solidified with 1.5% agar in culture dishes. These dishes were placed in an artificial climate chamber under conditions of 25°C in the light (18 h) and 20°C in the dark (6 h). After 11 to 12 weeks, archegonia had developed on the lower surface of the gametophytes just behind the growing apex.

Gametophytes bearing various archegonial stages were placed in 3% glutaraldehyde in 0.1 mol/L phosphate buffer at room temperature for 6-12 h. The specimens were subsequently washed three times with the same buffer, postfixed in 2% aqueous osmium tetroxide for 2 h, rinsed three times in buffer and embedded in Spurr's resin (SPI-Chem, USA) via a graded acetone series. Specimens were thick sectioned for the presence of the archegonia and thin-sectioned with a diamond knife on an ultramicrotome (Leica EM UC7, Germany). The thin sections were stained with uranyl acetate and lead citrate. All specimens were observed with a transmission electron microscope (Tecnai G2 Spirit BioTWIN, FEI Company).

RESULTS

Young Egg

Archegonia of *Diplopterygium glaucum* are produced on the lower surface just behind the growing point of the gametophyte. At the time of egg formation, the archegonium contains an axial row of three cells, *i.e.*, an egg cell, a ventral canal cell (VCC), and a single elongated neck canal cell (NCC) (Fig. 1a). The inner three cells are closely appressed to the archegonial jacket cells (Fig. 1a).

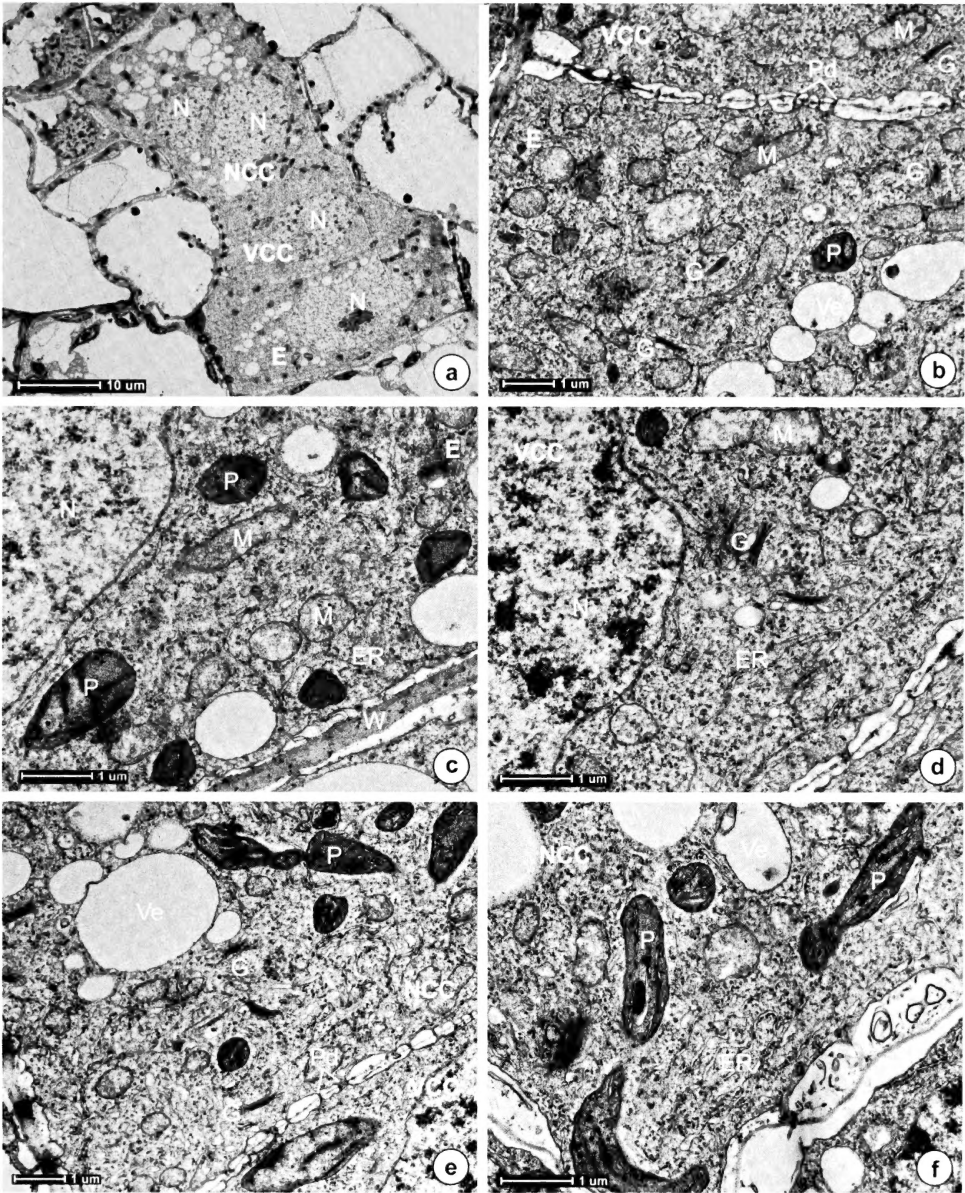


FIG. 1. YOUNG EGG STAGE OF *DIPLOPTERYGIUM GLAUCUM*. (a) A young archegonium, containing a newly formed egg (E), a ventral canal cell (VCC) and a neck canal cell (NCC) with two nuclei. (b) Part of the egg and the VCC showing plasmodesmata (pd) between the two cells, abundant Golgi bodies (G), vesicles (Ve) in the upper part of the egg cytoplasm. (c) Lower part of the egg showing thick wall between the egg and the jacket cell, abundant cytoplasmic organelles. (d) Lateral part of the VCC showing abundant Golgi bodies. (e) Lower part of the VCC, showing abundant Golgi bodies and other organelles. (f) Upper part of the VCC, showing abundant endoplasmic reticulum (ER), M, mitochondrion; N, nucleus; P, plastid.

There are well developed plasmodesmata between the egg and the VCC (Fig. 1b), and between the VCC and NCC (Fig. 1e), but no plasmodesmata were observed between the inner cells and the jacket cells (Fig. 1c). The nucleus of the egg is ellipsoid in shape and usually contains an irregular nucleolus (Fig. 1a). Vesicles, with a diameter of 0.5-1 μ m, are frequent in the egg cytoplasm (Fig. 1a-c). Plastids contain fewer lamellae and no starch grains (Fig. 1c). Mitochondria are distributed throughout the cytoplasm of the egg. In the VCC and NCC, there are more Golgi bodies in the cytoplasm (Fig. 1 d-e). Endoplasmic reticula are frequent in the periphery of the NCC cytoplasm (Fig. 1 f).

Maturing Egg

FORMATION OF THE SEPARATION CAVITY AND PORE REGION.—Soon after the egg is formed, a separation cavity forms between the egg and the ventral canal cell (Fig. 2 a, b). However, a connecting region (pore region) still links the egg and the VCC in the central region (Fig. 2a). There are well developed plasmodesmata in the pore region (Fig. 2c). An obvious wall, lying closely to the VCC, separates the egg and the VCC (Fig. 2a-c). Some amorphous materials can be seen in the separation cavity (Fig. 2b). At this stage, the Golgi bodies in the egg cytoplasm increase in number especially around the periphery of the egg (Fig. 2d). Some of the mitochondria beneath the pore region become elongated (Fig. 2c). Plastids are similar to the previous stage. There are many vesicles in the cytoplasm of the egg (Fig. 2a, b). Sometimes, vesicles fuse with the separation cavity (Fig. 2b). In the canal cells, ER and Golgi bodies are more abundant than before (Fig. 2e, f). Plasmodesmata continually connect the VCC and NCC during the development of the egg (Fig. 2e).

THE FORMATION OF THE EGG ENVELOPE AND THE FERTILIZATION PORE.—At the later stage of egg development, the VCC separates from the egg in the pore region (Fig. 3a). An egg envelope is formed in the upper surface of the egg (Fig. 3a). Higher magnification observation shows that the egg envelope is formed by opaque materials deposited on the dark stained plasmalemma (Fig. 3b). However, no egg envelop is formed in the pore region (Fig. 3b). The only membrane covering the fertilization pore is the plasmalemma (Fig. 3b). The diameter of the fertilization pore reaches about 3.5 μ m (Fig. 3b). Concentric membranous vesicles are seen beside the pore region (Fig. 3a, b, arrows). At this stage, the nucleus becomes irregular (Fig. 3b). Abundant vesicles and Golgi bodies are mainly located in the central part of the egg cytoplasm above the nucleus (Fig. 3a, b).

Mature Egg

When the egg matures, a layer of egg envelope covers the surface of the egg apart from the fertilization pore (Fig. 3c). The egg envelope in the upper surface of the egg is especially prominent. The thickness of the upper egg envelope decreases from the center to the periphery (Fig. 3c, d). In the lower part of the egg, the egg envelope is much thinner compared to the upper layer of the egg envelope (Fig. 3c). There are electron-light spaces inside the egg

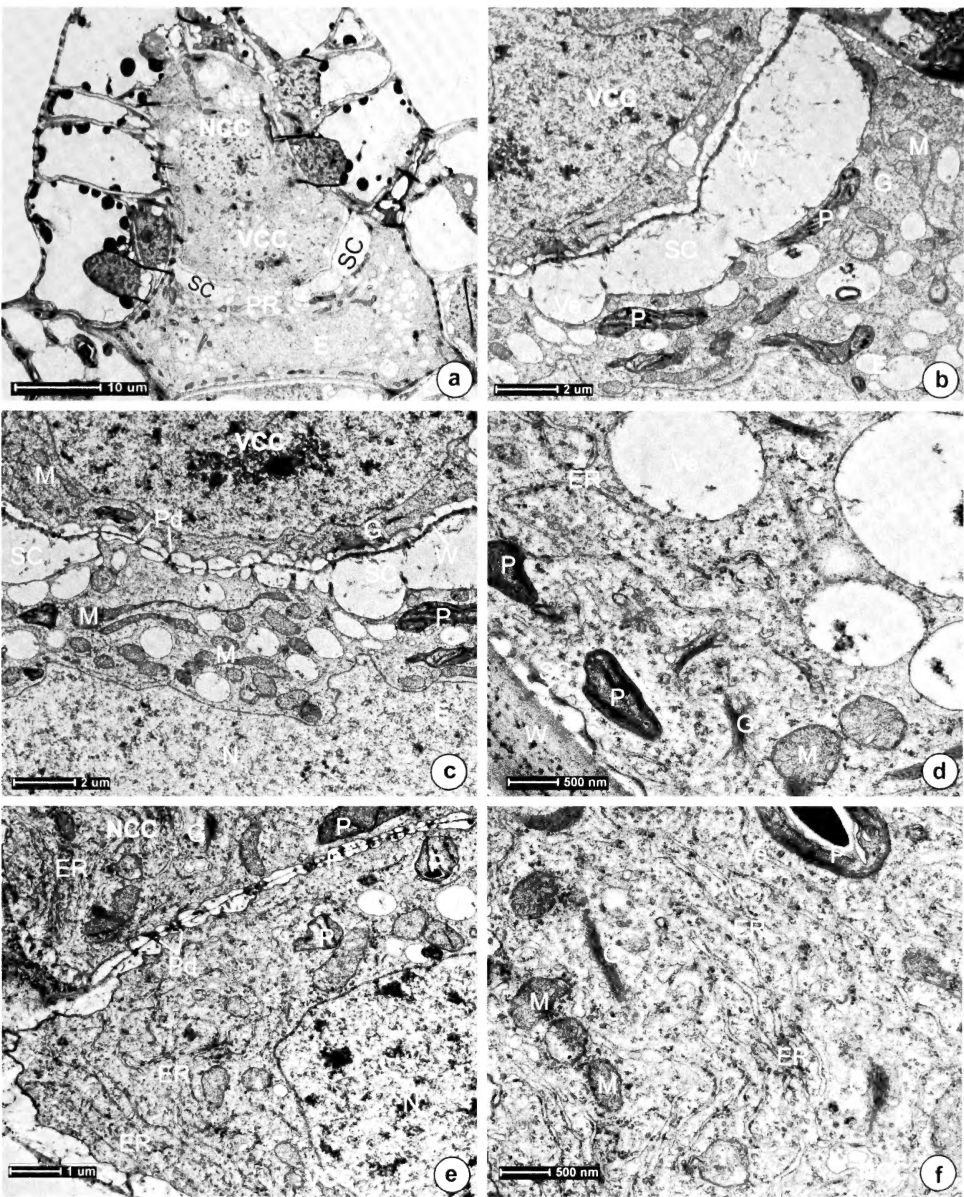


FIG. 2. MATURING EGG STAGE OF *DIPLOPTERYGIUM GLAUCUM*. (a) A separation cavity (SC) forms above the egg. A pore region (PR) connects the egg (E) and the VCC. (b) Higher magnification of Fig. 2a, a cell wall (W) lies close to the VCC. Vesicles of the egg present in separation cavity (SC). (c) Magnification of the pore region (PR) showing plasmodesmata (Pd) in the pore region. Mitochondria (M) are abundant beneath the pore region. (d) Lateral part of the egg showing Golgi bodies. (e) Part of the VCC and NCC showing abundant endoplasmic reticulum (ER). (f) Golgi bodies (G) and ER become more abundant in NCC. G, Golgi bodies; M, mitochondrion; N, nucleus; S, starch.

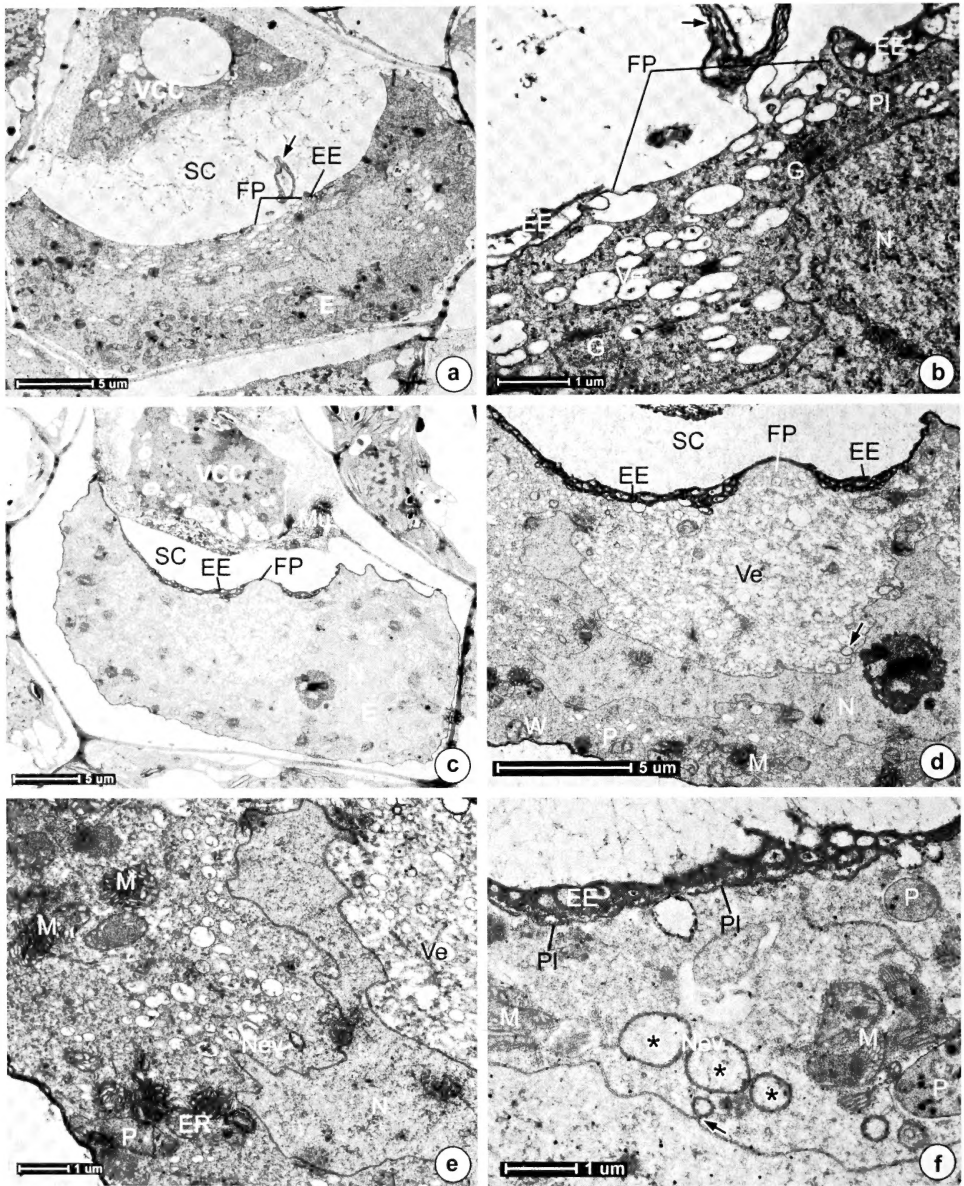


FIG. 3. MATURING AND MATURE EGG STAGE OF *DIPLOPTERYGIUM GLAUCUM*. (a) A fertilization pore (FP) and egg envelope (EE) form at the late stage of egg (E) development. (b) Magnification of the fertilization pore (FP), abundant vesicles (Ve) form in the upper part of the egg; c. A mature egg with a fertilization pore (FP). (d) Magnification showing vacuolation of the egg cytoplasm. Nuclear evaginations (arrow) form. (e) The nucleus of the egg becomes irregular in outline with nuclear evaginations (Nev). (f) Higher magnification showing the egg envelope and nuclear evaginations. M, mitochondrion; N, nucleus; VCC, ventral canal cell; Ve, vesicles.

envelope, resulting in a network structure in section (Fig. 3d). Higher magnification observation shows that the egg envelope is associated closely with the dark stained plasmalemma (Fig. 3f).

The nucleus of the egg remains highly irregular in outline and produces numerous sac-like evaginations near the main body of the nucleus (Fig. 3d, arrow; Fig. 3e, f). Some evaginations remain connected to the main body of the nucleus via narrow isthmuses (Fig. 3f, arrow). Sac-like evaginations, which, in this plane of section, are not connected to the nucleus, are frequently seen in the cytoplasm of the egg (Fig. 3f, asterisks). The matrix of these evaginations resembles that of the main body of the nucleus (Fig. 3f). However, the double membranes of the evaginations cannot be identified clearly (Fig. 3f). It is notable that the cytoplasm, surrounded by the cup-shaped nucleus in the upper part of the egg, is highly vacuolated (Fig. 3d). At this stage, plastids contain no starch grains or lamellae. Plastids can only be recognized by the presence of the plastoglobuli (Fig. 3f). The mitochondria possess well-developed cristae. At the last stage of egg maturation, the canal cells degenerate completely. Almost all of the cytoplasm of the canal cells has decomposed into amorphous mucilaginous materials (Fig. 3c).

DISCUSSION

The Egg Envelope and Fertilization Pore

Since the discovery of the fertilization pore in *Ceratopteris thalictroides* (Cao, Yang, and Wang, 2009), we have been dedicated to expanding the number of species for which egg development has been ultrastructurally studied to examine whether the fertilization pore exists in ferns in different evolutionary lineages. So far, it has been confirmed that all core-leptosporangiate ferns examined possess the egg envelope and fertilization pore in their mature eggs (Cao, Yang, and Wang, 2009; Cao, Wang, and Bao, 2010a; Cao *et al.*, 2010b, Cao, Dai, and Wang, 2011, 2012b; Wang *et al.* 2012a, b; Yang *et al.* 2013; Zou *et al.* 2014). Among the basal leptosporangiate ferns, *Osmunda japonica* and *Lygodium japonicum* have been investigated. Neither of the two species formed an egg envelope or fertilization pore (Cao, Dai, and Wang, 2012a; Cao *et al.*, 2017). The present investigation shows that *D. glaucum*, a gleichenioid fern, which current phylogenetic analyses (PPG I, 2016; Shen *et al.*, 2017; Smith *et al.*, 2006) demonstrate is a more ancient lineage than *L. japonicum*, has formed a typical egg envelope and fertilization pore. The diameter of the fertilization pore of *D. glaucum* (about 3.5 μm) is obviously larger than those of core-leptosporangiate ferns, whose fertilization pores are usually about 2.5-3.0 μm (Cao, Yang, and Wang, 2009; Cao *et al.*, 2010b, Cao, Dai, and Wang, 2011, 2012b; Wang *et al.* 2012a,b; Yang *et al.* 2013; Zou *et al.* 2013). The larger fertilization pore may be an ancestral feature in fern oogenesis.

The present investigation shows that the egg envelope of *D. glaucum* is reticulate in structure, which is the same as that found in the Cyatheaales and most members of the Polypodiales, including *Pteridium* (Bell and Mühlethaler, 1962a, b; Cao, Dai, and Wang, 2012b), *Athyrium filix-femina* (Fasciati *et al.*,

1994), *Dryopteris crassirhizoma* (Bao *et al.*, 2005), *Plagiogyria euphlebia* (Cao, Dai, and Wang, 2011), *Anisocampium shearerii* (Yang *et al.*, 2013), and *Phymatosorus hainanensis* (Zou *et al.*, 2014), but differs from the egg envelopes of the family of Pteridaceae *sensu lato* (Smith *et al.*, 2006), including *Ceratopteris* (Cao, Yang, and Wang, 2009; Cao, Yang, and Bao, 2010a; Lopez-Smith and Renzaglia, 2008), *Adiantum flabellulatum* (Cao *et al.*, 2010b) and *Coniogramme emeiensis* (Wang *et al.*, 2012a). Members of the Pteridaceae *sensu lato* usually possesses multilayered egg envelopes, which are considered to be formed by attachment of the endoplasmic reticulum (Cao, Yang, and Wang, 2008; Cao, Wang, and Bao, 2010a; Cao *et al.*, 2010b). The formation of the egg envelope and fertilization pore appears to prevent polyspermy (Cao *et al.* 2010c). However, the two types of the egg envelopes of the ferns may have phylogenetic implication. The basal leptosporangiate fern, *D. glaucum* shares a structure similar to most core-leptosporangiate ferns, but differs from that of Pteridaceae *sensu lato* indicating that *D. glaucum* shares this feature with the core-leptosporangiate ferns examined to date except Pteridaceae *sensu lato*.

Nuclear Evaginations and the Cytoplasmic Organelles

The present investigation demonstrates that *D. glaucum* forms nuclear evaginations in the mature egg stage of oogenesis. From previous observations, it has been shown that *Lygodium japonicum* also produces nuclear evaginations during oogenesis (Cao *et al.*, 2017). However, the fern *Osmunda japonica* (Cao, Dai, and Wang, 2012a), a clade at the base of the leptosporangiate ferns, produces no nuclear evaginations during oogenesis. Almost all core-leptosporangiate ferns also produce nuclear evaginations during oogenesis, including *Adiantum flabellulatum*, *Plagiogyria euphlebia*, *Pteridium aquilinum*, *Coniogramme emeiensis*, *Cibotium barometz*, *Phymatosorus hainanensis*, and *Anisocampium shearerii* (Cao *et al.* 2010b; Cao, Dai, and Wang, 2011; Cao, Dai, and Wang, 2012b; Cao *et al.*, 2017; Wang *et al.*, 2012a, b; Yang *et al.*, 2013; Zou *et al.*, 2014). Nuclear evaginations are considered to be relevant to the information exchange between nucleus and cytoplasm, and are important for the latter sporophytic growth (Bell and Duckett, 1976). However, the ferns (*Osmunda* and *Equisetum*) without nuclear evaginations during oogenesis can produce sporophytes as well (Cao, Dai, and Wang, 2012a; 2015). Therefore, the biological significance of forming nuclear evaginations still needs to be explored.

The cytoplasmic organelles of *D. glaucum* are basically similar to those of the core leptosporangiate ferns (Cao, Yang, and Wang, 2009; Cao, Wang, and Bao, 2010a; Cao *et al.*, 2010b; Cao, Dai, and Wang, 2011; 2012b; Cao *et al.*, 2017; Wang *et al.* 2012a, b; Yang *et al.* 2013; Zou *et al.* 2013). However, vacuolation of the cytoplasm, especially in the upper part of the egg, is scarcely seen in other leptosporangiate ferns.

The Characteristics of Oogenesis and Their Phylogenetic Significance

Current hypotheses of fern phylogeny are based on a combination of morphological evidence and nucleic acid sequence data (PPG I, 2016; Shen *et al.*, 2017; Smith *et al.* 2006). The morphological evidence of some sporophytic reproductive structures (such as the annulus and stomium) have been considered to be among the most conserved and consistent characters in classification and phylogeny (Shen *et al.*, 2017; Smith, 1995). For the most part, anatomical aspects of gametophytic reproductive structures have not been included in constructing phylogenetic hypotheses primarily because of the technical challenges involved in observing cellular details. Our previous observations from the basal groups to the higher groups of leptosporangiate ferns have demonstrated that the characteristics of fern oogenesis are similarly conserved within orders, but vary between orders (Cao, Yang, and Wang, 2009, Cao, Wang, and Bao, 2010a, Cao *et al.*, 2010b, Cao, Dai, and Wang, 2011, 2012b; Cao *et al.* 2017; Wang *et al.* 2012a, b; Yang *et al.* 2013; Zou *et al.* 2014). Thus, the evidence to date indicates that fern oogenesis may contribute to understanding phylogenetic relationships among clades. The gleichenioid fern *D. glaucum*, which is considered ancestral to the schizaeoid ferns according to the phylogenies based on molecular data (PPG I, 2016; Shen *et al.*, 2017; Smith *et al.*, 2006), has formed a typical egg envelope and the fertilization pore like the core-leptosporangiate lineage. Furthermore, the similar structure of the egg envelope and fertilization pore of *D. glaucum* and most members of the core-leptosporangiate ferns except members of the Pteridaceae *sensu lato* may indicate a closer relationship between gleichenioid fern and the core-leptosporangiate ferns (cf., Bierhorst, 1971; Holttum, 1973; Lovis, 1977) or may show that these features developed independently in the two lineages.

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The Alpine Ferns of the Trans-Mexican Volcanic Belt

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ABSTRACT.—Alpine vegetation is one of the most restricted ecosystems in Mexico. Its greatest extent occurs on the high peaks of the Trans-Mexican Volcanic Belt (TMVB). In this study we document fern diversity of the alpine vegetation of central Mexico, generate a floristic list, provide a key for determination, and report the elevational limits of the species present. The latter information will serve as a baseline for future comparisons to determine potential upward migration of fern species, as is predicted with global warming. In comparison to the páramo vegetation of Central and South America, fern diversity in the alpine vegetation of central Mexico is surprisingly low, and only five families, 11 genera, and 12 species are documented. This represents 1.5% of the ferns species known from the country. Polypodiaceae is the most diverse family, with four genera and four species, followed by Pteridaceae (three genera and three species) and Dryopteridaceae (two genera and two species). Aspleniaceae and Woodsiaceae each have a single genus, the former with two species and the latter with one species. All of the alpine ferns growing in the TMVB are leptosporangiate, and there are no arborescent, epiphytic, aquatic, or heliophilous ferns. *Asplenium castaneum*, *Cystopteris fragilis*, and *Polystichum speciosissimum* are widespread in the alpine vegetation, whereas the remaining species are restricted to one or few sites. With regard to upper elevational limit, the highest occurring species were *Asplenium castaneum* (4,569 m), *Polystichum speciosissimum* (4,490 m), and *Cystopteris fragilis* (4,377 m).

KEY WORDS.—fern diversity, global warming, Mexico, Neotropics, Polypodiaceae

There are approximately 10,578 species of ferns in the world (PPG I, 2016), and nearly a third of them (3,250 spp.) occur in the New World (Moran, 2008). Fern communities are richest in wet tropical regions, particularly in cloud forests (Kessler, 2010). Mexico is one of the most diverse countries in the world for fern species, and Mickel and Smith (2004) report 902 species from the country, whereas Villaseñor (2016) lists 932 species. Since this latter publication, at least one additional species has been described (Sundue, 2017). Together with Brazil and the Andean region of South America, Mexico is considered to represent one of the major centers of fern diversification in the Americas (Tryon, 1972). Ferns grow in all of the types of vegetation present in

Mexico (Rzedowski, 2006). The greatest richness has been registered in cloud forests (approx. 630 spp.), followed by tropical deciduous forests (536 spp.), and then tropical rainforests (438 spp.) (Tejero-Díez, Torrez-Díaz and Gual-Díaz, 2014). They are also distributed in structurally less complex ecosystems such as grasslands; however, they are much less diverse and a mere 21 species have been reported (Tejero-Díez, Torrez-Díaz and Gual-Díaz, 2014). Most of the diversity is in mountainous areas with a tropical climate, where they occur across broad elevational and climatic ranges.

Alpine vegetation is one of the most restricted ecosystems in Mexico. It occurs above timberline at the summits of the highest and coldest mountains. This is usually $\pm 3,900$ m, but can be as low as 3,600 m in northeastern Mexico and on El Ajusco. According to Rzedowski (2006), the Mexican alpine regions have mean annual temperatures from 3 to 5°C and can experience frosts throughout the year. Average rainfall varies from 600 to 800 mm, and high insolation coupled with strong winds result in high evaporation. Alpine vegetation occurs in Chiapas (Miranda, 1952) and various locations along the Sierra Madre Oriental in the states of Coahuila, Nuevo León y Tamaulipas (Beaman and Andresen, 1966; McDonald, 1990). It has also been reported for Oaxaca (McDonald, 2013) and Chihuahua (McDonald, Martínez, and Nesom, 2011), but these latter two sites are probably better considered as subalpine. Certainly, the greatest extent of alpine vegetation in Mexico is found in the Trans-Mexican Volcanic Belt (TMVB), an active volcanic zone that extends east-west across central Mexico from the state of Veracruz to the states of Jalisco and Nayarit (Gómez-Tuena, Orozco-Esquivel, and Ferrari, 2007). Within this region, alpine vegetation is restricted to 11 of the highest mountains: Nevado de Colima (NC), Nevado de Toluca (NT), El Ajusco (AJ), Iztaccíhuatl (IZ), Popocatepetl (PP), Cerro Telapón (TE), Monte Tlalóc (TL), La Malinche (LM), Pico de Orizaba, including Cerro Chichimeco (PO), Sierra Negra (SN), and Cofre de Perote (CP) (Steinmann *et al.*, unpublished data).

Eleven species of ferns have been previously reported from the alpine grasslands of the TMVB (Almeida-Leñero *et al.*, 2007; Arreguín-Sánchez, Fernández, and Quiroz, 2004; Matuda, 1956; McVaugh, 1992; Mickel and Smith, 2004; Narave, 1985). However, there is no synthesis of fern diversity across the region, and some of the mountaintops remain poorly explored (e.g., Sierra Negra and Tlalóc). Therefore, the purpose of this study is to document fern diversity of the alpine vegetation of central Mexico, generate a floristic list, provide a key to their determination, and report the elevational limits of the species present. The latter information will provide a baseline to allow future comparisons and determine potential upward migration of fern species, as is predicted to occur with global warming.

MATERIALS AND METHODS

Bimonthly field trips to the highest mountains of the TMVB were conducted over the course of two years (September 2012 to August 2014); sporadic visits have occurred since then. We followed Beaman's (1962) concept of timberline,

and only open areas above timberline are included in our study; grasslands occurring at lower elevations in openings within otherwise continuous forest were not considered. The preparation and collection of herbarium specimens followed the techniques presented by Lot and Chiang (1986). The specimens were generally gathered from elevations of 3,900 m and higher. Longitude and latitude coordinates, as well as altitude, were obtained from a GPS Garmin eTrex-30. In addition to the fieldwork, the collections of the following herbaria were examined: ENCB, FMCE, IEB, MEXU, MSC, NY, UAMIZ, and XAL (Thiers, cont. updated). A dichotomous key for the determination of the TMVB alpine fern species was prepared using diagnostic features visible on herbarium specimens. Classification and species circumscription follow Mickel and Smith (2004), Sigel *et al.* (2014), and PPG I (2016). Voucher specimens collected during the study were deposited in IEB.

RESULTS

Five families, 11 genera, and 12 species are here reported from the alpine grasslands of the TMVB (Table 1). Nine of these species were found during this study. Only *Alansmia spathulata* (A.R. Sm.) Moguel & M. Kessler, *Asplenium fibrillosum* Pringle & Davenp., and *Melpomene peruviana* (Desv.) A.R. Sm. & R.C. Moran were not recollected, but they are known by previous collections from the Cofre de Perote or Iztaccíhuatl, at elevations of 3,950–4,150 m. Polypodiaceae is the most diverse family, with four genera and four species. This is followed by Pteridaceae (three genera and three species) and Dryopteridaceae (two genera and two species). Aspleniaceae and Woodsiaceae each have a single genus, the former with two species and the latter with one species. There are three new records for the alpine vegetation of the TMVB: *Pleopeltis polylepis* (Roem. ex Kunze) T. Moore from the ecotone with *Pinus hartwegii* Lindl. forests on Popocatepetl (4,089 m) and *Myriopteris lendigera* (Cav.) J. Sm. and *Pellaea ternifolia* (Cav.) Link from the Sierra Negra (4,237 m) where they grew together under the shade of large rocks in an area with scattered individuals of *Pinus hartwegii*.

Of the fern species previously reported from the alpine vegetation of the TMVB, *Elaphoglossum mathewsii* (Fée) T. Moore is probably the taxon that was mentioned as simply *Elaphoglossum* sp. for the alpine vegetation of Iztaccíhuatl by Almeida-Leñero *et al.* (2007). Although we did not encounter this species during our fieldwork on Iztaccíhuatl, it was previously documented from there, as well as the Cofre de Perote and Sierra Negra. Two additional species included in Mickel and Smith (2004) have upper elevational limits that suggest they occur in alpine vegetation: *Dryopteris pseudofilix-mas* (Fée) Rothm. (3,950 m) and *Melpomene moniliformis* (Lag. ex Sw.) A.R. Sm. & R.C. Moran (4,000 m). However, despite our fieldwork and an extensive search in herbaria, we have been unable to locate any specimens that confirm their presence in the alpine grasslands. *Polypodium californicum* Kaulf. was reported from the alpine vegetation of the Cofre de Perote (Mickel and Smith 2004), but this report was corrected to *Polypodium calirhiza* S.A.

TABLE 1. Diversity, geographical range and sampling dates of ferns of the FVTM alpine belt, * = new records. Site abbreviations: AJ, El Ajusco; CF, Cofre de Perote; IZ, Iztaccíhuatl; LM, La Malinche; NC, Nevado de Colima; NT, Nevado de Toluca; PO, Pico de Orizaba; PP, Popocatepetl; TE, Cerro Telapón; TL, Monte Tláloc; SN, Sierra Negra. Country abbreviations: Arg, Argentina; Bol, Bolivia; Braz, Brazil; Col, Colombia; CR, Costa Rica; Ec, Ecuador; ES, El Salvador; GA, Greater Antilles; Guat, Guatemala; Hisp, Hispaniola; Hond, Honduras; JF, Juan Fernandez Islands; Mex, Mexico; Nic, Nicaragua; Pan, Panama; USA, United States of America; Ven, Venezuela.

Family	Species	FVTM sampling location and elevation (m)	Geographical distribution
Aspleniaceae	<i>Asplenium castaneum</i>	AJ 3,750; CF 3,930-4,040; IZ 3,900-4,200; LM 4,082; NC 4,000-4,100; NT 4,175-4,300; PO 3,950-4,377; PP 3,850-4,082; SN 4,271-4,569; TL 4,000-4,034	Central and southern Mex; Guat, CR, Pan, Col, Ven, Ec, Peru, Bol
Dryopteridaceae	<i>Asplenium fibrillosum</i>	IZ 3,900	Central Mex
	<i>Elaphoglossum mathewsii</i>	CF 3,930; IZ 3,800-4,100; SN 4,282 m	Central and southern Mex; Guat, CR, Col, Ven, Ec, Peru, Bol, Chile
	<i>Polystichum speciosissimum</i>	AJ 3,745; CF 3,930-4,267; IZ 3,940-3,950; LM 3,929; NT 4,140; SN 4,370; PO 4,086-4,490; PP 3,963	Central and southern Mex; Guat, CR, Pan
Polypodiaceae	<i>Alansmia spathulata</i>	CF 3,950; IZ 3,990-4,150	Central and southern Mex; Guat
	<i>Melpomene peruviana</i>	CF 3,930; IZ 4,100; TL 4,000	Central and southern Mex; Guat, Hond, CR, Pan, Col, Ven, Sur, Ec, Peru, Bol, Braz
	<i>*Pleopeltis polylepis</i>	PP 4,089	Northern and central Mex; USA, Guat
Pteridaceae	<i>Polypodium calirhiza</i>	CF 3,930-4,025	Central Mex; USA
	<i>*Myriopteris lendigera</i>	SN 4,237	USA, Mex, Guat, Hond, Nic, CR, Pan, Hisp, Col, Ven, Ec
	<i>Gaga marginata</i>	AJ 3,660	Mex, Guat, Hond, ES, CR, Pan, Col, Ven, Ec, Peru, Bol, Arg
	<i>*Pellaea ternifolia</i>	SN 4,237	USA, Mex, Central and South America, Hawaii

TABLE 1. Continued.

Family	Species	FVTM sampling location and elevation (m)	Geographical distribution
Woodsiaceae	<i>Cystopteris fragilis</i>	CF 3,930-4,171; IZ 3,800-4,200; LM 4,082; NC 3,990; NT 4,020; PO 3,950-4,377; PP 3,962-4,082; TE 4,079; TL 4,000-4,034; SN 4,282	USA, Mex, Guat, Hond, ES, Nic, CR, Pan, GA, Col, Ven, Ec, Peru, Braz, Bol, Chile, JF, Arg, Europe, Asia, Africa, Hawaii

Whitmore & A.R. Sm. (Sigel *et al.*, 2014). Also, Lehnert (2007) treats the Mexican alpine plants of *Melpomene*, as *M. peruviana*, instead of *M. pilossisima* (M. Mart. & Gal.) A.R. Sm. & R.C. Moran as has been previously reported.

Asplenium castaneum Schltdl. & Cham. occurred in 10 of the 11 sites surveyed, *Cystopteris fragilis* (L.) Bernh. in nine sites, *Polystichum speciosissimum* (A. Braun ex Kunze) Copel. in eight sites, *Elaphoglossum mathewsii* and *Melpomene peruviana* in three, *Alansmia spathulata* in two, and *Asplenium fibrillosum*, *Myriopteris lendigera*, *Gaga marginata* (H.B.K.) F.W. Li & Windham, *Pellaea ternifolia*, *Pleopeltis polylepis*, and *Polypodium calirhiza* were found in a single site (Table 1). The alpine areas with the highest fern diversity were the Cofre de Perote and Iztaccíhuatl, each with seven species, and the Sierra Negra with six species. These were followed by El Ajusco, La Malinche, Monte Tláloc, Nevado de Toluca, and the Pico de Orizaba with three species. Popocatepetl and the Nevado de Colima had two species, whereas Cerro Telapón was the least diverse with a single species. With regard to upper elevational limit, the highest occurring species were *Asplenium castaneum* (4,569 m), *Polystichum speciosissimum* (4,490 m), and *Cystopteris fragilis* (4,377 m), and all of these limits were reached along the mountainous chain of the Pico de Orizaba-Sierra Negra in the eastern portion of the TMVB (Table 1).

All fern species registered by us grow in the cracks of rocky outcrops mainly in moist micro-habitats. Only *Asplenium castaneum* and *Cystopteris fragilis* (Fig. 1) occur in moist microhabitats of protected areas between rocks as well as dry sites. They are also the most widely distributed species of the alpine grassland and occur both in low and high alpine zones. Finally, we collected six species in the low alpine zone: *Pleopeltis polylepis* was found in the ecotone with *Pinus hartwegii* forest at Popocatepetl, whereas *Myriopteris lendigera*, *Elaphoglossum mathewsii*, *Pellaea ternifolia* were encountered at the Sierra Negra and *Polypodium calirhiza* at Cofre de Perote growing under the shade of large rocks in an area with scattered individuals of *Pinus hartwegii*.

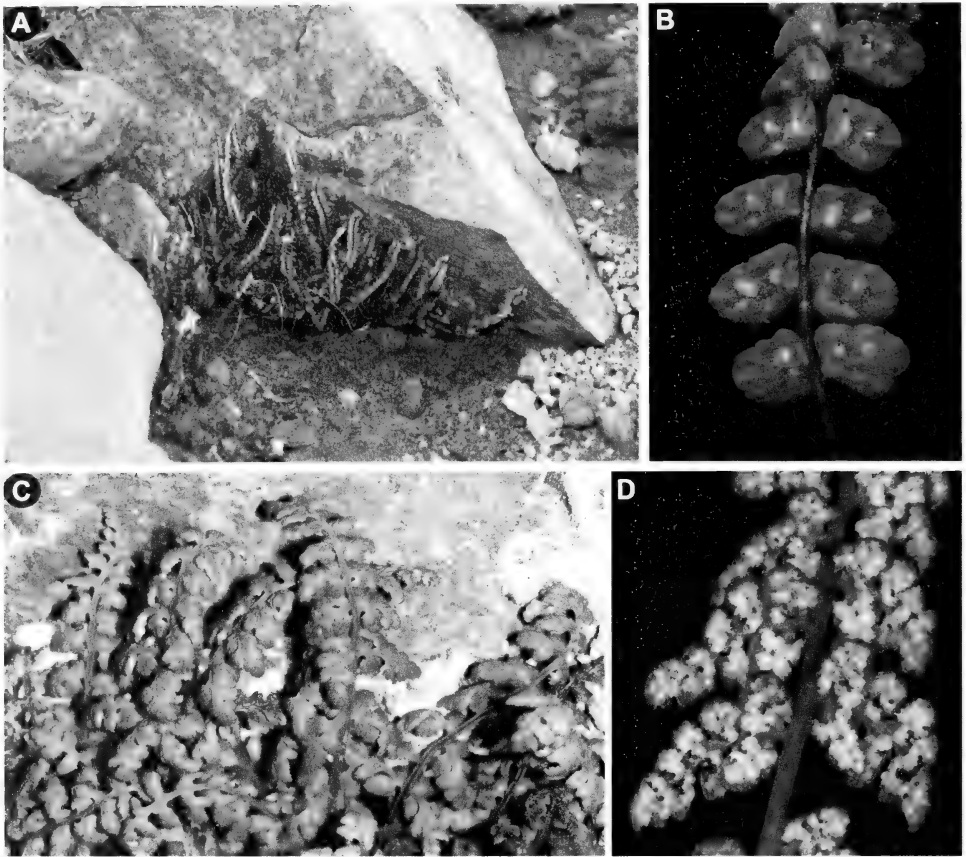


FIG. 1. Habit and close up of the fronds of *Asplenium castaneum* and *Cystopteris fragilis*. A. Habitat of *Asplenium castaneum*. B. Close up of *Asplenium castaneum*. C. *Cystopteris fragilis*. D. Close up of *Cystopteris fragilis* (Photographs by Victor Steinmann).

DISCUSSION

Depending on the origin and features of the sporangium, ferns are divided into two groups: eusporangiate and leptosporangiate. The leptosporangiate ferns reach their greatest diversity in tropical regions but also are present in temperate forests and arid zones. They can be terrestrial, epiphytic, hemi-epiphytic, aquatic, or subaquatic. Their size varies from diminutive plants a few centimeters tall, such as species of *Azolla* Lam., to arborescent forms of *Cyathea* Sm. and *Dicksonia* L'Hér. that exceed 10 meters tall (Mendoza-Ruiz and Ceja-Romero, 2014). All of the alpine ferns growing in the TMVB are leptosporangiate, low statured, and with small pinnae. The families present are among the 10 most diverse fern families in Mexico, in particular the three most species-rich families Pteridaceae, Dryopteridaceae, and Polypodiaceae (Mickel and Smith, 2004). Although Aspleniaceae and Woodsiaceae are the least diverse families, each with a single species, their representatives,

Asplenium castaneum and *Cystopteris fragilis*, respectively, are the most widely distributed and abundant ferns in the alpine grasslands surveyed. No epiphytic, arborescent, or aquatic ferns occur in the area, and in general, the alpine ferns of the TMVB grow in the cracks of isolated, rocky outcrops.

Only one alpine fern species is endemic to Mexico (*Asplenium fibrillosum*), whereas in total there are about 190 fern species endemic to the country (Mickel and Smith, 2004). Thus, the proportion of endemism in the alpine vegetation regions is lower than the national average (8% vs. 20%). None of the species reported here are strictly alpine, and they all descend to at least 3,000 m, with some occurring at elevations of less than 1,500 m (Mickel and Smith, 2004), see Fig. 2. All of the non-endemic species also occur in alpine areas outside of the country. In Mexico, *Asplenium castaneum* has the highest distribution; it was found at 4,569 m on the Pico de Orizaba. This species was previously known from up to 4,000 m in the region of Iztaccíhuatl-Popocatepetl and nearly 4,200 m at the summit of the Nevado de Colima (Almeida-Leñero *et al.*, 2007; Arreguín-Sánchez, Fernández, and Quiroz, 2004; McVaugh, 1992). *Cystopteris fragilis* has been found at 4,377 m on the Pico de Orizaba. This species is noteworthy for holding the world record for the highest occurring fern and has been found growing at 5,485 m in Chang La, Ladakh, India (Webster, 1961). It is also considered one of the most phenotypically plastic and wide-ranging species in the world, and it occurs in numerous mountainous ecosystems with a wide range of altitudes and temperatures (Kessler, 2010). These three species are also the most abundant and widespread species in the TMVB.

The alpine vegetation of Central and northern South America is commonly called páramo, and in contrast to Mexico, South America has vast expanses of alpine vegetation and timberline commonly occurs as low as 3,000 m; it is also more humid (Luteyn, 1999). These páramos by far house the greatest diversity of alpine plants in the world (Barrington, 2005; Luteyn, 1999), and alpine ferns similarly reach their highest diversity there (Tryon and Tryon, 1982), with Sklenar *et al.* (2005) reporting 20 families, 50 genera, and 345 species. In comparison, the alpine vegetation of Mexico is poor in ferns, and of its approximately 930 species, only approximately 1.5% occur in alpine habitats. Seven species are shared between the páramos of South America and the alpine regions of the TMVB (Table 1). One of these, *Elaphoglossum mathewsii*, is noteworthy for occurring up to 5,100 m in South America (Luteyn, 1999), but in Mexico it occurs to 4,282 m. *Melpomene peruviana* also reaches a considerably higher elevation in South America, 5,200 m as compared to 4,100 m (Lehnert, 2007). This species is also remarkable because it holds the world's record for the highest elevation vascular epiphyte, recorded at 4,542 m (Sylvester *et al.*, 2014). However, in Mexico it is not known to be epiphytic (Lehnert, 2007).

Eleven species are shared with the highlands of Central America (Table 1). In contrast, only three species extend northward into the United States. Two of the latter are very widespread and also occur in the páramos of South America. Thus, the affinities of the alpine fern flora of México are clearly with the alpine

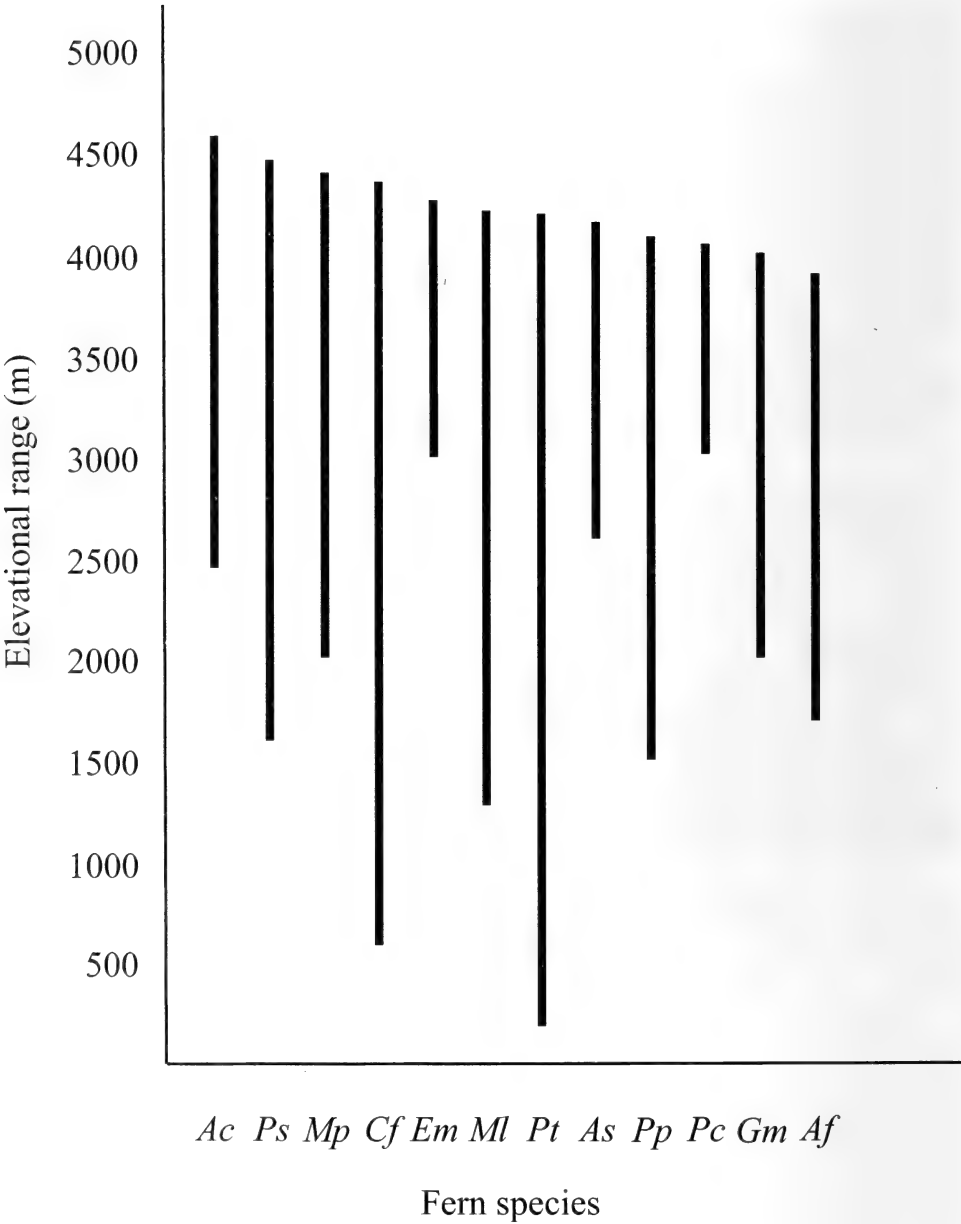


FIG. 2. Comparison of elevational ranges of the fern species under study. Ac, *Asplenium castaneum*; Af, *Asplenium fibrillosum*; Em, *Elaphoglossum mathewsii*; Ps, *Polystichum speciosissimum*; As, *Alansmia spathulata*; Mp, *Melpomene peruviana*; Pp, *Pleopeltis polylepis*; Ml *Myriopteris lendigera*; Gm, *Gaga marginata*; Pt, *Pellaea ternifolia*; Cf, *Cystopteris fragilis*.

neotropical vegetation of Central and South America and not Nearctic. However, considering that ferns in general are diverse in tropical regions this southerly affinity is not surprising.

There are no studies focusing on the ferns in other alpine areas of Mexico, but judging by reports in the available literature, they are even more poorly represented than in the TMVB. García-Arévalo and González-Elizondo (1991) did not encounter any fern species in their survey of alpine regions of Cerro Potosí. Farther to the south, Pérez-Ferrera (pers. comm., 2018) reports only three alpine fern species in his checklist of the Tacaná Volcano in Chiapas: (*Asplenium castaneum*, *Polystichum speciosissimum*, and an undetermined species of *Cheilanthes*).

In contrast with other Neotropical alpine zones, for example the Costa Rican páramo (Barrington, 2005), the alpine belt of the TMVB harbors no arborescent, epiphytic, aquatic, or heliophilous ferns, and in general, the alpine ferns grow in moist, often shady cracks of rocky outcrops. It is interesting to note that both *A. castaneum* and *C. fragilis* occupy from moist to xeric microhabitats in the TMVB alpine belt, so their success in the alpine region is probably due to characteristics that allow them to survive xeric conditions.

Additionally, one question to consider is whether the diversity of ferns in the alpine belt of the TMVB is increasing due to the rise of species due to global warming, as has been registered for other groups of plants (Steinbauer *et al.*, 2018). However, there is not a sufficient number of records of specimens of herbarium or literature data that allow comparisons.

The information presented here will permit future comparisons with the current diversity and elevational limits of the Mexican alpine ferns. Such studies are important because they can be used to detect potential changes in the face of global warming (e.g., Pouteau *et al.*, 2016). However, clearly more fieldwork is desirable for the alpine areas outside of the TMVB and there are many questions to be addressed within the region. For example, compact growth forms and morphological characteristics such as buried rhizomes, thick fronds with rounded segments, as well as thick indument formed by the scales have been correlated with the frigid environments that occur in high tropical mountains (Barrington, 2005; Kluge and Kessler, 2007; Tryon and Tryon, 1982). Because most of the Mexican alpine ferns have wide elevational distributions, it would be interesting to compare morphological variation across various ecosystems in order to determine if plants from alpine habitats differ from those growing in less extreme conditions and how. Furthermore, it would be beneficial to analyze the diversity of Mexican ferns across gradients from lower elevations and latitudes to higher elevations and latitudes to compare abundance and diversity on a much broader scale.

KEY TO THE ALPINE FERNS OF THE TRANS-MEXICAN VOLCANIC BELT.

1. Base of the petiole with two vascular bundles; sori with an indusium 2
1. Base of the petiole with one, three or more vascular bundles; sori without an indusium . . 7

2. Petiole straw-colored to red-brown; fronds pinnate-pinnatifid; indusium cup-shaped or ovate *Cystopteris fragilis*
2. Petiole brown, black-purple or blackish; fronds 1-many times pinnate; indusium submarginal, elongate 3
3. Sori submarginal, elongate; indusium entire, fibriate or papillate 4
3. Sori on veins, elongate; indusium entire to suberose 6
4. Pinnae ternate *Pellaea ternifolia*
4. Pinnae not ternate 5
5. Petiole dark castaneous, terete, with orange hairs *Myriopteris lendigera*
5. Petiole castaneous, grooved, glabrous *Gaga marginata*
6. Indusia conspicuously ciliate or laciniate *Asplenium fibrillosum*
6. Indusia entire to crenulate or somewhat erose, but not ciliate *Asplenium castaneum*
7. Fronds simple to pinnatifid 8
7. Fronds once to various times pinnate 10
8. Fronds pinnatisect to pinnatifid, not dimorphic; rhizome scales concolorous, brown; petiole and rachis glabrous *Polypodium calirhiza*
8. Fronds simple, dimorphic; rhizome scales bicolorous, to clathrate or red-brown to black; petiole and rachis pubescent 9
9. Fronds dimorphic; rhizome scales red-brown to black; sori acrostichoid *Elaphoglossum mathewsii*
9. Fronds not dimorphic; rhizome scales bicolorous to clathrate; sori rounded. *Pleopeltis polylepis*
10. Fertile portion of the frond 2-pinnate to 2-pinnate-pinnatifid; rhizome scales concolorous; petiole and rachis with scales; sori with a false indusium, arranged submarginally *Polystichum speciosissimum*
10. Fertile portion of the frond lobed to pinnate; rhizome scales bicolorous to clathrate and iridescent; petiole and rachis with setae; sori otherwise 11
11. Rhizome scales conspicuously clathrate; sori 4–7 per pinna; sporangia with setae *Melpomene peruviana*
11. Rhizomes scales not or scarcely clathrate; sori 1–3 per pinna; sporangia glabrous. *Alansmia spathulata*

EXSICCATE.

Alansmia spathulata (A.R. Sm.) Moguel & M. Kessler

SPECIMENS EXAMINED.—**COFRE DE PEROTE:** Veracruz, Mpio. Perote, 3,950 m, *Narave 823* (MEXU). **IZTACÍHUATL:** Puebla, south side of Iztacíhuatl, ca. 7 km north of Paso de Cortes, 4,150 m, *Beaman 2876* (MSC).

Asplenium castaneum Schltdl. & Cham.

SPECIMENS EXAMINED.—**COFRE DE PEROTE:** Veracruz, Mpio. Perote, ladera suroeste del volcán Cofre de Perote (19°29'31.4"N, 97°08'59.4"O), 4,123 m, *Hernández-Cárdenas & Arredondo-Amezcuca 1526* (IEB). **EL AJUSCO:** Ciudad de México, Parque Nacional Cumbres del Ajusco, cordillera oeste, ruta que sube desde el albergue alpino (19°12'52"N, 99°15'54"O) 3,750 m, *Rincón & Morales 4011* (IEB). **IZTACÍHUATL:** Estado de México, south side of Iztacíhuatl, 4,000–4,500 m, *Beaman 1962* (MSC). **LA MALINCHE:** Tlaxcala, Mpio. Huamantla, ladera norte del volcán Matlalcueye (La Malinche) (19°14'7.05"N, 98°2'7.9"O), 4,082 m, *Hernández-Cárdenas & Arredondo-Amezcuca 1578* (IEB). **MONTE TLÁLOC:** Estado de México, Mpio. Ixtapaluca, ladera S del Monte Tláloc (19°24'36.5"N,

98°42'53.9"O), 4,034 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1150 (IEB). **NEVADO DE COLIMA:** Jalisco, Mpio. Zapotlán el grande (Cd. Guzmán), ladera noroeste del Nevado de Colima, camino al picacho (19°33'41.3"N, 103°36'38.1"O), 4,063 m, *Hernández-Cárdenas & Arredondo-Amezcu* 800 (IEB). **NEVADO DE TOLUCA:** Estado de México, Mpio. Toluca, en la base de la cima rocosa del Pico del Águila, ladera de pendiente pronunciada y de fragmentos medianos, cara SO del Nevado de Toluca, 4,300 m, *Madrigal & González MAGT* 499 (IEB). **PICO DE ORIZABA:** Veracruz, Mpio. La Perla, ladera sureste del volcán Pico de Orizaba (Citlaltépetl) (19°00'08.7"N, 97°16'13.1"O), 4,377 m, *Hernández-Cárdenas et al.* 1721 (IEB). **POPOCATÉPETL:** Estado de México, Mpio. Amecameca, ladera norte del Volcán Popocatepetl a medio kilómetro del antiguo albergue Tlamacas (19°03'8.6"N, 98°38'7.9"O), 4,082 m, *Hernández-Cárdenas & Arredondo-Amezcu* 836 (IEB). **SIERRA NEGRA:** Puebla, Mpio. Atzitzintla, ladera NE del volcán Tliltépetl (Sierra Negra) (18°59'10.4"N, 97°18'47.6"O), 4,569 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1014 (IEB).

Asplenium fibrillosum Pringle & Davenp.

SPECIMENS EXAMINED.—**IZTACCÍHUATL:** Estado de México, Mpio. Amecameca, La Joya de Alcalican, extremo SW del Iztaccíhuatl, 3,900 m, *Hernández* 15/78-117 (IEB).

Myriopteris lendigera (Cav.) J. Sm.

SPECIMENS EXAMINED.—**SIERRA NEGRA:** Puebla, Mpio. Atzitzintla, ladera noreste de la Sierra Negra (Tliltépetl), saliéndose a los 2.5 km del camino que lleva al GTM, partiendo de la pluma (18°58'55.0"N, 97°18'15.5"O), 4,237 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1734 (IEB).

Cystopteris fragilis (L.) Bernh.

SPECIMENS EXAMINED.—**CERRO TELAPÓN:** Estado de México, Mpio. Ixtapaluca, cima del cerro Telapón (19°22'15.6"N, 98°43'12"O), 4,079 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1245 (IEB). **COFRE DE PEROTE:** Veracruz, Mpio. Perote, ladera suroeste del volcán Cofre de Perote (19°29'17.1"N, 97°09'3.7"O), 4,171 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1547 (IEB). **IZTACCÍHUATL:** Estado de México, alpine zone on SW slope of Volcán Iztaccíhuatl, 5-6 km N of Paso de Cortes, supur of Cerro Amacuilecatl, above and NE of La Joya shelter (ca. 19°08'15"N, 98°38'40"W), 4,050 a 4,200 m, *Barkley et al.* 1038a (MSC). **LA MALINCHE:** Tlaxcala, Mpio. Huamantla, ladera norte del volcán Matlalcueye (La Malinche) (19°14'7.05"N, 98°2'7.9"O), 4,082 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1580 (IEB). **MONTE TLÁLOC:** Estado de México, Mpio. Ixtapaluca, ladera S del Monte Tláloc (19°24'36.5"N, 98°42'53.9"O), 4,034 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1135 (IEB). **NEVADO DE COLIMA:** Jalisco, Mpio. Zapotlán el Grande (Cd. Guzmán), ladera noroeste del Nevado de Colima, camino

al picacho (19°33'47.8"N, 103°36'51.5"O), 3,990 m, *Hernández-Cárdenas & Arredondo-Amezcu* 810 (IEB). **NEVADO DE TOLUCA:** Estado de México, Mpio. Tenango del Valle, pendiente sobre barranca, en la ladera E del volcán Nevado de Toluca, casi en los límites del bosque, 4,020 m, *Eugenia & Héctor MAGT* 427 (IEB). **POPOCATÉPETL:** Estado de México, Mpio. Amecameca, ladera norte del volcán Popocatepetl a medio kilómetro del antiguo albergue Tlamacas (19°03'8.6"N, 98°38'7.9"O), 4,082 m, *Hernández-Cárdenas & Arredondo-Amezcu* 837 (IEB). **PICO DE ORIZABA:** Puebla, Mpio. Atzitzintla, ladera suroeste del volcán Pico de Orizaba (Citlaltépetl) (19°00'27.5"N, 97°17'14.9"O), 4,291 m, *Hernández-Cárdenas et al.* 1472 (IEB). **SIERRA NEGRA:** Puebla, Mpio. Atzitzintla, ladera NE del volcán Tliltépetl (Sierra Negra) (18°58'57.9"N, 97°18'21.9"O), 4,282 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1035 (IEB).

Elaphoglossum mathewsii (Fée) T. Moore

SPECIMENS EXAMINED.—**COFRE DE PEROTE:** Veracruz, east side of Cofre de Perote, 3,930 m, *Beaman* 2175 (MEXU, MSC). **IZTACCÍHUATL:** Estado de México, ladera W del Iztaccíhuatl, arriba de El Salto, Valle de Ayoloco, 4,000 a 4,100 m, *Rzedowski* 23469 (MSC). **SIERRA NEGRA:** Puebla, Mpio. Atzitzintla, ladera noreste de la Sierra Negra (Tliltépetl), saliéndose a los 2.5 km del camino que lleva al GTM, partiendo de la pluma (18°59'10.6"N, 97°18'18.9"O), 4,282 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1742 (IEB).

Gaga marginata (H.B.K.) F.W. Li & Windham

SPECIMEN EXAMINED.—**EL AJUSCO:** Ciudad de México, Parque Nacional Cumbres del Ajusco, cordillera E, ruta que sube de Santo Tomás Ajusco (19°12'46"N, 99°14'41"O), 3,660 m, *Rincón & Cervantes* 4072 (UAMIZ).

Melpomene peruviana (Desv.) A.R. Sm. & R.C. Moran

SPECIMENS EXAMINED.—**COFRE DE PEROTE:** Veracruz, east side of Cofre de Perote, 3,930 m, *Beaman* 2173 (MSC). **IZTACCÍHUATL:** Estado de México, south side of Iztaccíhuatl, near La Joya, 4,100 m, *Beaman* 3575 (MSC). **MONTE TLÁLOC:** Estado de México, (19°24'N, 98°43'O), 4,000 m, *Wendt & Atkinson* (ENCB, NY).

Pellaea ternifolia (Cav.) Link

SPECIMENS EXAMINED.—**SIERRA NEGRA:** Puebla, Mpio. Atzitzintla, ladera noreste de la Sierra Negra (Tliltépetl), saliéndose a los 2.5 km del camino que lleva al GTM, partiendo de la pluma (18°58'55.0"N, 97°18'15.5"O), 4,237 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1733 (IEB).

Pleopeltis polylepis (Roem. ex Kunze) T. Moore

SPECIMEN EXAMINED.—**POPOCATÉPETL:** Estado de México, Mpio. Amecameca, ladera norte del Volcán Popocatepetl en el antiguo albergue Tlamacas (19°03'30.3"N, 98°38'7.32"O), 4,089 m, *Hernández-Cárdenas & Arredondo-Amezcu* 841 (IEB).

Polypodium calirhiza S.A. Whitmore & A.R. Sm.

SPECIMEN EXAMINED.—**COFRE DE PEROTE:** Veracruz, Mpio. Perote. Cofre de Perote, 8.5 km al sur de El Conejo, sobre el camino a las torres (19°29'50"N, 97°09'06"O) 4,025 m, *Ramírez-Amezcu & Steinmann* 2011 (IEB).

Polystichum speciosissimum (A. Braun ex Kunze) Copel.

SPECIMENS EXAMINED.—**COFRE DE PEROTE:** Veracruz, north side of Cofre de Perote, 4,267 m, *Stoutamire* 2774 (MSC). **EL AJUSCO:** Ciudad de México, Parque Nacional Cumbres del Ajusco, cordillera central, ruta que sube al Pico del Águila (19°12'52"N, 99°15'22"O), 3,745 m, *Rincón & Gómez* 4041 (IEB). **IZTACCÍHUATL:** Estado de México, La Joya de Alcalican extremo SW del Iztaccíhuatl, 3,950 m, *Rzedowski* 25398 (MSC). **LA MALINCHE:** Tlaxcala, Mpio. Huamantla. Ladera norte del volcán Matlalcueye (La Malinche) (19°14'25.4"N, 98°01'56.3"O), 3,929 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1114 (IEB). **NEVADO DE TOLUCA:** Estado de México, shore of large lake in the crater Nevado de Toluca, 4,140 m, *Beaman* 1884 (MSC). **POPOCATÉPETL:** Estado de México, Mpio. Amecameca, ladera norte del volcán Popocatepetl, Tlamacas (19°03'29.16"N, 98°38'7.08"O), 3,963 m, *Hernández-Cárdenas & Arredondo-Amezcu* 2048 (IEB). **PICO DE ORIZABA:** Puebla, Mpio. Atzitzintla, ladera suroeste del volcán Pico de Orizaba (Citlaltépetl) (19°00'39.9"N, 97°16'48.1"O), 4,490 m, *Hernández-Cárdenas et al.* 1451 (IEB). **SIERRA NEGRA:** Puebla, Mpio. Atzitzintla, ladera este del volcán Tliltépetl (Sierra Negra) (18°59'3.41"N, 97°18'30.2"O), 4,370 m, *Hernández-Cárdenas & Arredondo-Amezcu* 1566 (IEB).

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Population Structure and Habitat Characteristics of *Dicksonia sellowiana* Hook. in the Sierra Madre Oriental, Mexico

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ABSTRACT.—The American tree fern *Dicksonia sellowiana* reaches its northern distribution limit in Mexico. This species grows only in the highly fragmented remnants of the montane cloud forest (MCF). Given the lack of data on ecological aspects of this endangered tree fern species, considered under special protection by Mexican legislation, the objectives of the present research were to investigate the structure of *D. sellowiana* populations and to characterize its habitats. The study was carried out in seven populations located at different elevations and latitudes in the MCF of the Sierra Madre Oriental in Mexico. In each locality, three sampling plots were established to characterize the habitat and to record density, height, stem diameter, number of leaves, and individual fertility. The resulting values were analyzed with univariate and multivariate statistics. In four of the *D. sellowiana* populations, individuals of low height predominated; these were classified as dynamic populations. However, the density of individuals by unit area was low compared with some South American populations, probably because of the high degree of fragmentation of the MCF. The variation in population density was mainly explained by the vegetation cover. Environmental factors such as elevation and slope orientation, however, explained better the variation of the basal area of tree fern populations. These results constitute the first reference on the current state of Mexican *D. sellowiana* populations, which is important for the management and conservation of this endangered species, and for monitoring changes in the environmental conditions of its natural habitat, the MCF of Mexico.

KEY WORDS.—Disturbance, extinction risk, montane cloud forest, population ecology, tree ferns.

The genus *Dicksonia* comprises 26 tree fern species (PPG-I, 2016; Noben *et al.*, 2017), of which 5 are exclusive to the Neotropics, but only *D. sellowiana* Hook. has a wide distribution (Ulloa-Ulloa *et al.*, 2017a). The latitudinal distribution limit of this species in the northern hemisphere is in eastern and southern Mexico, in the states of Chiapas, Guerrero, Hidalgo, Oaxaca, Puebla,

and Veracruz (Villaseñor, 2016). However, it is in Central and South America (Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Peru, and Venezuela) where *D. sellowiana* populations are the most dense and most widely distributed (Ulloa-Ulloa *et al.*, 2017).

Geographic distribution and population structure are two basic characteristics that need to be taken into account to investigate and generate conservation and management strategies for plant species at risk (Schmitt and Windisch, 2006; Agurauja *et al.*, 2008; Mehltreter, 2010). It is therefore important to add to quantitative data on the current state of populations and habitat characteristics at the local and regional level (Mehltreter and García-Franco, 2008; Pérez-Paredes, Sánchez-González, and Tejero-Díez, 2014). Mexican populations of *D. sellowiana* grow exclusively in the montane cloud forest (MCF), a type of vegetation with a restricted distribution and a high degree of fragmentation (Eleutério and Pérez-Salicrup, 2009; Gual-Díaz and Rendón-Correa, 2014), characterized by having high humidity and moderate temperature conditions. It is estimated that the MCF currently covers 1% of Mexico, but of this area, only 0.4% is primary vegetation, and the remaining 0.6% is secondary vegetation (Gual-Díaz and Rendón-Correa, 2014), which is the reason why the MCF is at risk of disappearing and urgently requires action to be taken for its conservation and management (Arriaga *et al.*, 2000). Given this scenario, it is necessary to contribute data on the ecological aspects of plant species of the MCF.

The structure of a plant population can be described through the age, size, or stage of the life cycle of the individuals (Agurauja, Moora, and Zobel, 2004; Weber *et al.*, 2015). In arborescent ferns it is complex or even impossible to define the exact age of the individuals, and there is still controversy about the degree and type of relationship between fertility and the age of the plants (Mehltreter and García-Franco, 2008; Schmitt and Windisch, 2012; Pérez-Paredes, Sánchez-González, and Tejero-Díez, 2014). Therefore, the stem height of individuals (ontogenetic stage of the life cycle) continues to be a criterion widely used to describe populations in a single census. This method has proven to be very useful in a large number of population studies with different arborescent fern species, including *D. sellowiana* (Mehltreter and García-Franco, 2008; Schmitt, Schneider, and Windisch, 2009; Schmitt and Windisch, 2012; Marques and Kuprek, 2014; Pérez-Paredes, Sánchez-González, and Tejero-Díez, 2014; Weber *et al.*, 2015; Magna-Silva *et al.*, 2017; Mallmann *et al.*, 2018).

Individuals of *D. sellowiana* grow slowly and can live up to 200 years (Weber *et al.*, 2015); they reach heights of 5–10 m and have fronds up to 5 m long (Tryon and Tryon, 1982; Mickel and Smith, 2004). Since it is a perennial species with high density and biomass, this raises the environmental heterogeneity and favors the coexistence or inhibition of growth of other animal and plant species at its natural habitats, giving it a structural and functional role in the MCF (Blair *et al.*, 2017; Negrao *et al.*, 2017). Mexican populations of *D. sellowiana* are at risk, mainly due to habitat reduction; its trunk is harvested to obtain fiber (“maquique”) used as a substrate for growing

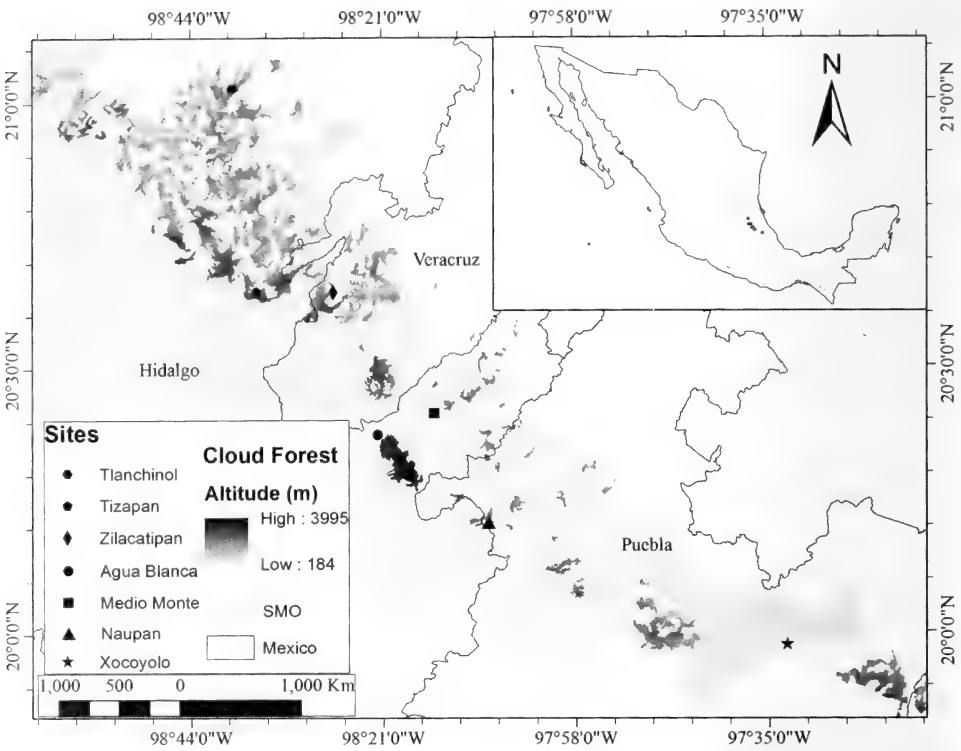


FIG. 1. Geographic locations of *D. sellowiana* populations in the central-south region of the Sierra Madre Oriental.

ornamental plants in the state of Veracruz (Muñiz-Díaz, Mendoza-Ruíz, and Pérez-García, 2007; Palacios-Rios, 2007), but in South America it is the tree fern species most used for this purpose (Palacios-Rios and Mehlreter, 1999; Mallmann *et al.*, 2018).

Dicksonia sellowiana populations show a wide distribution in the MCF, but they develop under precarious conditions in vegetation patches, isolated from each other because of fragmentation, which is why they are currently in the “special protection” category of the Mexican legislation (Semarnat, 2010) and their commercialization is regulated by international agreements (Cites, 2012). Given the lack of data on the basic ecological aspects of this species, the main objectives of this research were (1) to investigate the population structure of *D. sellowiana* and (2) to characterize its habitat in the Sierra Madre Oriental of Mexico.

MATERIAL AND METHODS

Study area.—*Dicksonia sellowiana* populations are distributed in the MCF of the Sierra Madre Oriental (SMOR) at elevations ranging from 900 to 2200 m (Fig. 1) on slopes of 30°–100°; the dominant climates are Cwa (temperate, dry

winter, and hot summer) in Agua Blanca, Medio Monte, Tizapan, Tlanchinol (Hidalgo), and Zilacatipan (Veracruz); Cwb (temperate, dry winter, and warm summer) in Naupan (Puebla) and Cfb (temperate, no dry season, and warm summer) in Xocoyolo (Puebla; Peel, Finlayson, and Macmahon, 2007). The average annual temperature at sampling sites ranges between 12 and 23°C, and the total annual precipitation between 1000 and 3000 mm, although there is a season of low rainfall (the sites have 0–4 months with few rainy days each year), which is reflected in foliage loss of some tree elements, but the MCF in its primary state maintains its foliage all year. Fog is frequent and atmospheric humidity is high, with the consequent decrease in luminosity (Rzedowski, 2006; Gual-Díaz and Rendón-Correa, 2014).

Field sampling.—Seven populations of *D. sellowiana* were analyzed, located at different latitudes in the MCF of the SMOR (Fig. 1). According to Hanski and Simberloff (1997), a local population is defined as a group of individuals found in the same habitat and sharing similar environmental conditions. From this definition, the criterion to consider them as populations was based on the presence of at least ten individuals in forest patches big enough to establish three sampling units under the canopy, each measuring 25×25 m (625 m^2 ; Álvarez-Zúñiga *et al.*, 2012; Pérez-Paredes, Sánchez-González, and Tejero-Díez, 2014). Schmitt, Schneider, and Windisch (2009) and Alfonso-Moreno *et al.* (2011), suggest a minimum area of 400 m^2 for the characterization of populations of tree fern species, so the size of the sampling plots in the present study was adequate. A total of 21 sampling units were established: three in each population, at least 20 m apart.

Density and coverage (basal area) are two important population attributes to define the role and ecological status of plant species. Changes occurring in both, under different environmental conditions, are of particular interest because of the implications for management and conservation of species considered at risk (Walker, Mehlreter, and Sharpe, 2010; Negrao *et al.*, 2017). In each sampling unit, density (every stem with a frond crown was considered to be an individual), stem height, stem diameter at 130 cm (DBH), number of mature and fertile individuals, and number of live and senescent leaves were recorded (Kingston, Waldren, and Smyth, 2004; Jones *et al.*, 2007). Individuals shorter than 1.30 m were measured at the basal part of the frond crown, without including the base of the petioles. DBH values were used to calculate the basal area of each individual based on the formula πr^2 and the total sum of individuals per population (Mateucci and Colma, 1982). The estimation of the relationship between stem height, stem diameter, and number of live and senescent leaves of the individuals was made with regression analysis; among the models generated (linear and non-linear), the one that presented the highest determination coefficient value (R^2) was chosen (Urban *et al.*, 2010).

Population structure.—Stem height values were used to classify populations as dynamic (Dy), normal/standard (No) or regressive (Re), based on the criteria (adapted and simplified) from Aguraiuja, Moora, and Zobel (2004) and Pérez-Paredes, Sánchez-González, and Tejero-Díez (2014): a dynamic population is characterized by the predominance of individuals of small size (in the present

study, this included all plants < 1.2 m in height); a normal or standard population is defined by the presence of individuals of all size classes, but with a predominance of intermediate heights (plants 1.2–4 m in height); and a regressive population is characterized by the dominance of tall individuals (>4 m in height) and a limited number of small and/or intermediate-sized ones. The morphological values of individuals (except density and basal area) in the different populations were contrasted with a one-way variance analysis, using Tukey's test for multiple comparisons of means, following a test of normality.

As an alternative strategy to define the type of population (dynamic, normal or regressive) in a more objective and robust way, the cluster analysis technique (CA) was used to identify morphological groups (Galván-Hernández *et al.*, 2018) based on height, stem diameter, and the number of vegetative leaves of individuals (the number of senescent leaves was not included in the CA, due to its high correlation with the number of vegetative leaves, $r = 0.74$, $P < 0.001$). For this purpose, Ward's method, considered to be a robust algorithm (Cao, Bark, and Williams, 1997; Singh, Hjørleifsson, and Stefansson, 2011) was used as a criterion for group clustering. The data were then analyzed using an inverse ANOVA to find the number of morphological groups that explained the total and intrinsic variation of the sample (the attributes of the species itself as a basis for class segregation). The clusters obtained were contrasted by k means to describe the significant differences among them (Stat Soft Inc., 2010).

Habitat characterization.—In each of the 21 sampling units, the habitat was characterized and the degree of disturbance determined by estimating the variables proposed by Pérez-Paredes, Sánchez-González, and Tejero-Díez (2014), modified for the conditions observed during fieldwork. The following variables were estimated: distance between sampling sites and human settlements (the values were obtained directly from Google Earth Pro, DIST); elevation (m, ELV) obtained with a Global Positioning System Garmin eTrex 10; terrain slope (degrees, SL), calculated with a Suunto clinometer; orientation (degrees, OR), recorded with a Suunto Tandem compass; percentage of vegetative ground cover (GVC) and percentage of rock cover (RC), using the Braun-Blanquet scale (1979); canopy cover (CC), estimated with a convex spherical model A densiometer (Lemmon, 1956); number of roads (trails [T], unpaved roads [UR], highways [H]) within each plot; felling or clearing (EXT) was estimated using a subjective scale, with values ranging from 1–10, divided into three intervals: 1–3 representing the lowest values, 4–6 the intermediate levels, and 7–10 the high levels; the presence of landslides (LS) was classified according to the area they occupied in m^2 (Appendix).

The degree of relationship between population density (or basal area values), and variable values obtained by habitat characterization was estimated through a non-linear analysis technique (regression trees; RT). RTs are very effective and useful for expressing non-linear relationships, since they do not require *a priori* assumptions about any type of behavior between the variables, and the interpretation of the results is simple, which is not the case with linear

statistical models (Sánchez-González and López-Mata, 2005; Kallimanis *et al.*, 2007; Krzywinski and Altman, 2017).

Recursive partitions of the population density values (or basal area values) from the 21 sampling sites were made using the RT algorithm, resulting in increasingly homogeneous subgroups. At each division, the values of the categorical or continuous environmental values used to characterize the habitat were selected as threshold values that resulted in the greatest reduction in the error sum of squares of population density (or basal area) in the sampling sites. The resulting RT structure has the following characteristics: it begins at a node (root), and continues through a series of binary divisions (stems), determined on the basis of values of the independent variables (used to characterize habitat) and ends at a series of terminal nodes (leaves). The number that appears at the start of the observations that follow the same route from the root to a particular leaf represents the arithmetic mean of the population density (or basal area) of a particular subset of *D. sellowiana* populations (Kallimanis *et al.*, 2007). All the analyses were calculated using the Statistica version 10 math program (Stat Soft Inc., 2010).

RESULTS

Characterization of population structure.—A total of 470 individuals were counted (3.6 individuals/100 m²), and population density in sample sites varied from 31–92 plants. The highest density of individuals was recorded at Tizapan (n=92), followed by Medio Monte and Agua Blanca with 86 and 70, respectively (Fig. 2).

The classification of population types based on individual size categories was similar to the classification obtained using cluster analysis criteria. The cluster analysis resulted in the identification of three significantly different categories or morphological groups in the populations (Table 1, Fig. 3). The *D. sellowiana* populations of Medio Monte, Tizapan, Xocoyolo, and Zilacatipan (Group 1) showed a high number of individuals (between 54 and 90%) in an early development class of less than 1.2 m in height, so they were classified as dynamic (Dy). The populations in Agua Blanca, Naupan, and Tlanchinol (Group 2) showed a predominance of intermediate class individuals (between 1.2 and 4 m height), with 78, 51 and 37%, respectively, so they were classified as normal (No).

The population in the municipality of Tlanchinol showed the highest mean DBH (31.48 ± 16.81), height (2.48 ± 1.77), number of green fronds (13.84 ± 8.07) and number of senescent fronds (13.45 ± 8.74). The lowest values of DBH (13.72 ± 7.88), number of vegetative leaves (5.70 ± 3.23) and number of senescent leaves (4.18 ± 3.70) were observed in the Xocoyolo population, and the lowest mean height at Tizapan (0.77 ± 1.0 ; Fig. 4).

There were significant statistical differences among populations ($P < 0.01$) in DBH, height, and number of green and senescent leaves (Fig. 4, Table 2). A highly significant positive correlation ($P < 0.001$) was found between height and diameter ($R=0.72$, $y=(0.17+0.03x)^2$); height and number of vegetative

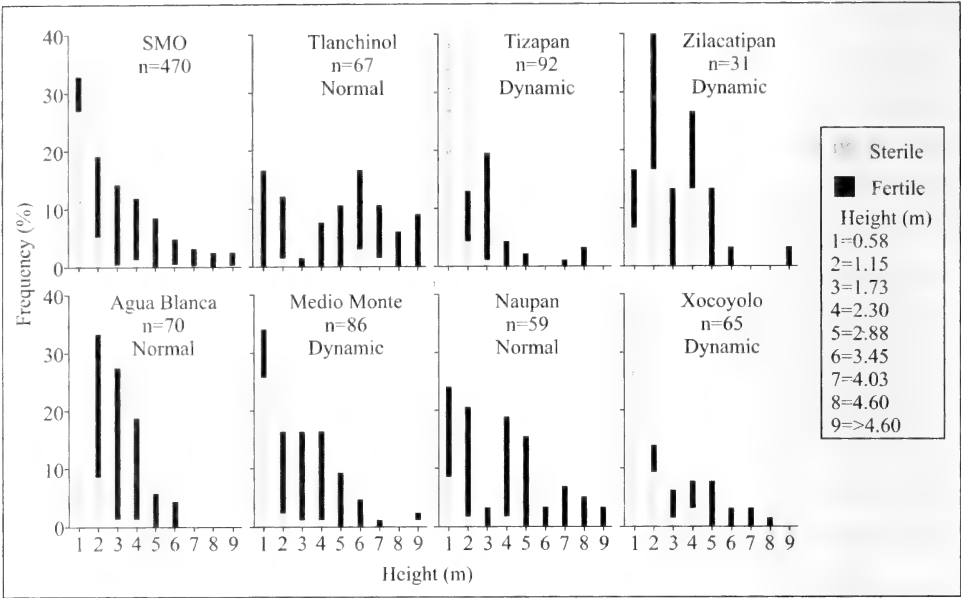


FIG. 2. *Dicksonia sellowiana* population types defined based on size structure (heights of individuals).

leaves $R=0.65$, $y=(3.14+6.25x)^2$; height and number of senescent leaves ($R=0.55$, $y=(2.61+6.16x)^2$); diameter and number of green leaves ($R=0.75$, $y=(1.5+0.06x)^2$); and between diameter and number of senescent leaves ($R=0.71$, $y=(1.05+0.07x)^2$).

Fertility.—Among the 470 individuals analyzed, 305 were fertile, which amounts to 64% of the total—the number of fertile individuals in each population varied from 24–59. The percentages of fertile individuals were: Naupan (88.1), Medio Monte (81.4), Tlachinol (77.6), Zilacatipan (77.4), Agua Blanca (74.3), Tizapan (46.7), and Xocoyolo (30.8). At Medio Monte, Naupan, Tizapan, Xocoyolo, and Zilacatipan some individuals with heights of 0.13–0.50 m (a mean of 18 cm in diameter) with developed sporangia were observed. Sterile individuals of more than 2 m in height were found in all populations (Fig. 2) except Tizapan, where all individuals ≥ 2 m were fertile.

TABLE 1. Morphological groups defined using cluster analysis. The specific classification criteria for *Dicksonia sellowiana* populations are indicated. Letters indicate statistical significant differences ($p < 0.001$) among groups obtained by k-means analysis ($F_{(2,468)} = 589.99$; $F'_{(2,468)} = 187.03$; $F''_{(2,468)} = 462.04$). DBH: Diameter at Breast Height. N: Number of individuals.

Morphological group	DBH	Height	Vegetative leaves	N
Small individuals	13.79±8.40 ^a	0.70±0.82 ^a	5.24±2.94 ^{a''}	259
Medium individuals	28.46±4.02 ^b	2.03±1.04 ^{b'}	14.17±4.42 ^{b''}	187
Large individuals	48.99±11.85 ^c	3.94±1.51 ^{c'}	21.20±5.42 ^{c''}	25

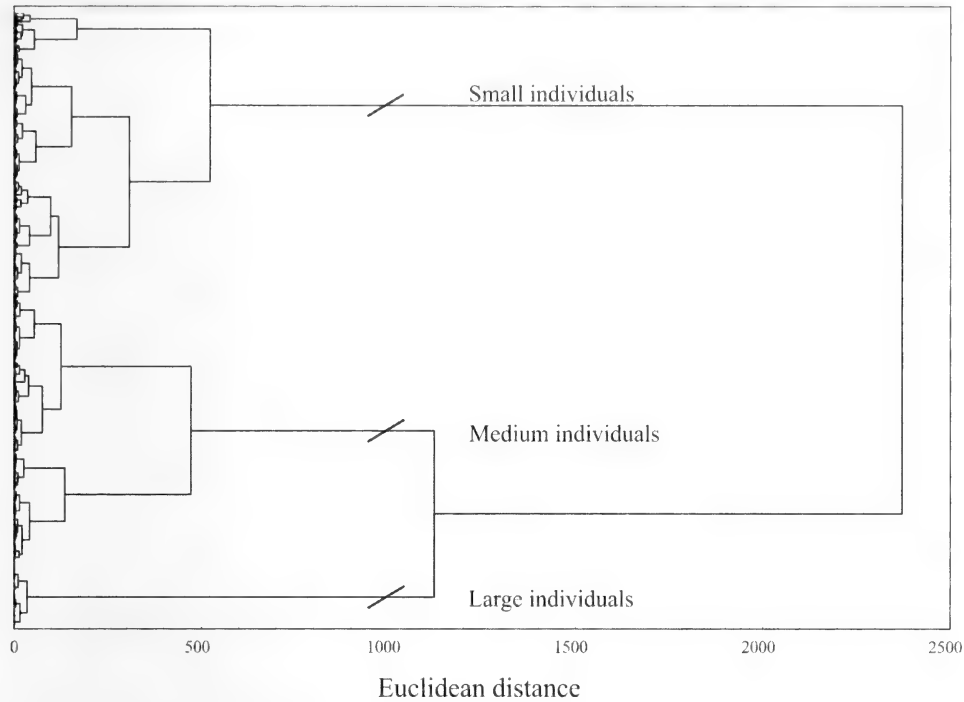


FIG. 3. Dendrogram of cluster analysis of *D. sellowiana* populations showing morphological groups identified on the basis of DBH (Diameter at Breast Height), height, and number of vegetative leaves.

Relationship between population density and habitat characteristics.—All populations of *D. sellowiana* found during the field trips were growing within the forest patches, but not at the periphery, so it was not necessary to consider the edge effect in the sampling design. The results of the regression tree indicate that ground vegetation cover (GVC), clearing or extraction (EXT), and distance between sampling sites and human settlements (DIS) are the variables most related to variations in *D. sellowiana* population density. The lowest number of individuals was found in the plots where GVC was less than 62.6% and the clearing rate was higher than 1.5; and the highest mean density of individuals occurred in sites with values of GVC above 62.6% and distances to human settlements less than 13.5 min (in one sampling plot) or less than 36 min (in four sampling plots) (Fig. 5).

Relationship between basal area (biomass) and habitat characteristics.—The regression tree indicates that elevation and slope orientation were the two variables that best explained variation in basal area of the populations. In the sense that the basal area tends to increase with elevation, except for three plots, corresponding to the population in the Tlanchinol municipality (1458.9 m a.s.l.), in which the mean BA value was higher than 1000 m²/ha at elevations below 1595 m. The basal area was larger in localities with a northwest exposure (Fig. 6).

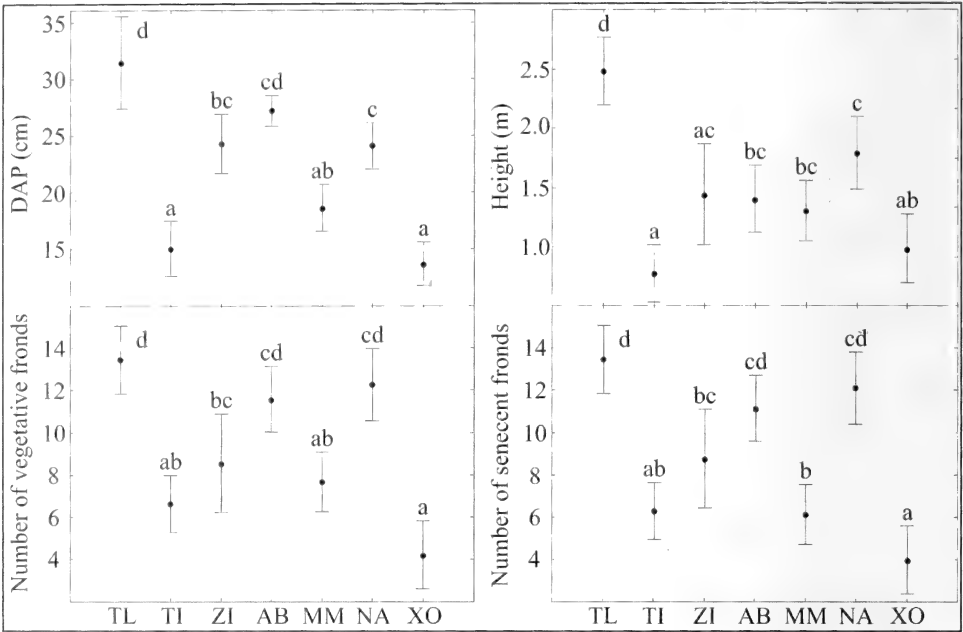


FIG. 4. Average values of attributes of *D. sellowiana* populations in Agua Blanca (AB), Naupan (Na), San Bartolo (SB), Tizapan (Ti), Tlanchinol (Tl), Xocoyolo (Xo) and Zilacatipan (Zi). Different letters indicate significant differences.

DISCUSSION

Density in populations of *D. sellowiana* is variable and differs by region: in Brazil, Gasper *et al.* (2011), Weber *et al.* (2015), Mallmann *et al.* (2018), Biondi *et al.* (2009), and Marques and Krupek (2014) counted 1.5, 1.5, 13.2, 13.3, and 13.8 individuals (average values per plot, standardized to 100 m²), respectively, and in Colombia, Alfonso-Moreno *et al.* (2011) recorded 14.2. The average number of *D. sellowiana* individuals per plot in SMOR was low (3.6 individuals /100 m²), compared to those recorded in some South American populations, and relatively similar to the values obtained for other species in Mexico such as *Alsophila firma* (Baker) D.S. Conant (8.1 individuals /100 m²)

TABLE 2. ANOVA test of four morphological characteristics of *D. sellowiana* populations in the Sierra Madre Oriental, Mexico. *P<0.001

Variable	Df	Sum Sq	Mean Sq	F value
Height	6	135.7	22.618	15.77*
DHB	6	18132	3022.1	28.17*
Vegetative fronds	6	3799	633.2	19.6*
Senescent fronds	6	4641	773.4	17.44*

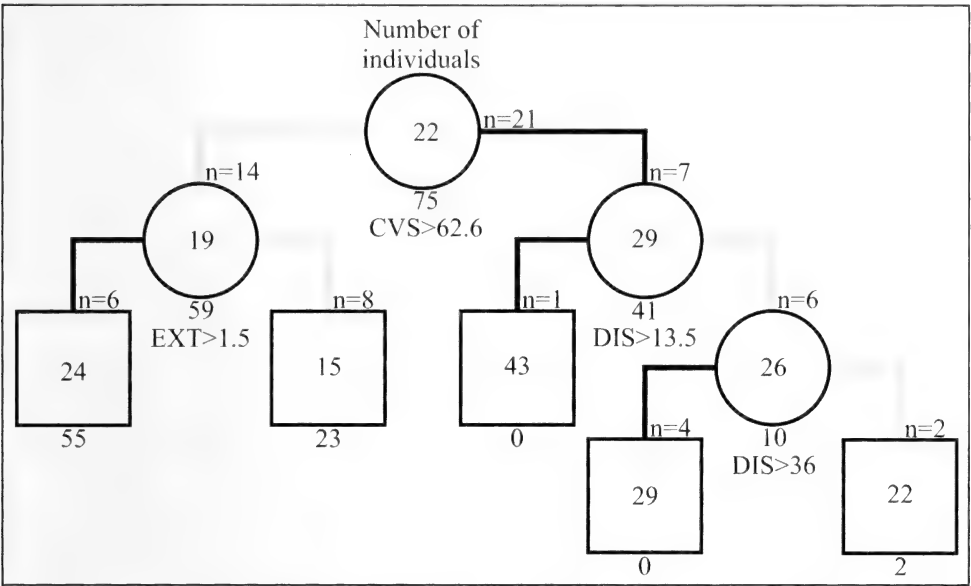


FIG. 5. Regression tree of population density in *D. sellowiana*. Circles and squares represent non-terminal and terminal nodes, respectively. Number in each circle or square indicates the mean number of individuals in the populations varying according to the branches of the tree towards a specific terminal node. Number under each circle or square indicates the sum of squares (associated with the arithmetic mean of all samples remaining through the nodes). Numbers in the links between nodes represent the decision criteria by which a particular environmental variable provides the basis for division. Solid black lines represent the route determining the highest population density, and gray lines the route determining the lowest population density. GVC. Ground vegetation cover; EXT. Clearing or extraction; DIS. Distance to the closest settlement.

and *Cyathea fulva* (M. Martens & Galeotti) Fée: 4.4/100 m² (Pérez-Paredes, Sánchez-González, and Tejero-Díez, 2014).

Variation in population density has been associated mainly with the degree of habitat disturbance (Marques and Krupek, 2014; Pérez-Paredes, Sánchez-González, and Tejero-Díez, 2014; Magna-Silva *et al.*, 2017). Consequently, the low individual density of *D. sellowiana* by unit area could be related to the increasingly fragmented and reduced distribution of MCF in Mexico, mainly caused by deforestation, farming, mining, and urban spread (Sánchez-Ramos and Dirzo, 2014). In contrast, other populations of large-sized ferns (*Lophosoria quadripinnata* (J.F. Gmel.) C. Chr.) and tree ferns (*Cyathea divergens* Kunze) are favored and show high density in disturbed environments (Eleutério and Pérez-Salicrup, 2009).

It has been suggested that the variation in population density of *D. sellowiana* is due to the intensity of light reaching the understory (Weber *et al.*, 2015), to humidity and edaphic conditions (Alfonso-Moreno *et al.*, 2011; Mallmann *et al.*, 2018), to low water availability and low anthropic activity (Marques and Krupek, 2014), and to low drought periods (Gasper *et al.*, 2011). One of the environmental factors most correlated to high individual density of

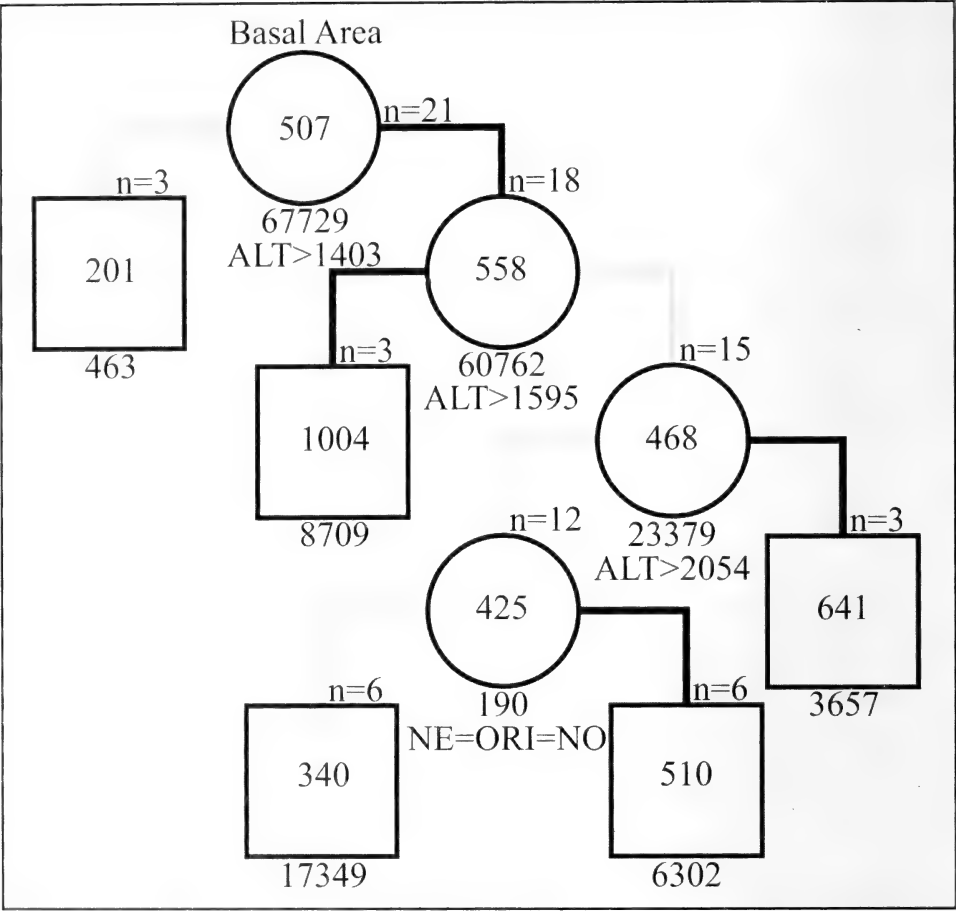


FIG. 6. Regression tree of basal area in *D. sellowiana*. As explained for FIG. 5, but here the dependent variable is mean population basal area. Vectors with black lines represent the route determining the largest basal area, and vectors with gray lines the route determining the lowest basal area. ELV. Elevation; NE. Northeast; NW. Northwest; ORI. Orientation.

D. sellowiana in SMOR was ground vegetation cover (GVC). It is probable that this variable is an indicator of local environmental conditions suitable for successful development of the species (under some degree of disturbance in the forest). Another factor related to population density was the distance between sampling localities and human settlements. Localities closest to settlements showed a high population density, which could seem contradictory since they would be expected to be more susceptible to human-caused disturbances. However, in Mexico most forests are property of private owners or “ejidatarios”, which is why the localities closest to towns are better supervised and those farther away are not. A good example is the Tlanchinol locality, where the owners of the forest routinely visit their lands, so it is difficult for other people to extract plants and cut down trees.

Another important factor to consider is the environmental history of the localities where *D. sellowiana* grows. In the Tlanchinol population, for example, where the most robust tree ferns are found, natural regeneration of the forest was common in the past, but in recent years, people clear or “weed” the trails they customarily use. In contrast, in the Naupan locality, forest fires are common and unknown people furtively extract entire small individuals and half the trunk of more robust individuals. In Xocoyolo the forest was fragmented and surrounded by pasture. In this locality craftsmen extract the “maquique” or “xaxim” (root fiber) of two species of arborescent ferns (*Alsophila firma* and *Cyathea fulva*), but not of *D. sellowiana*, since they claim that the trunk of this species does not have the necessary characteristics they need for their products. The only use of *D. sellowiana* observed in the present study was for sale as ornamental plant at traditional markets in the Sierra Norte in the state of Puebla.

An analysis of the structure of tree fern populations can be useful for recognizing patterns at a regional level (Agurauja, Moora, and Zobel, 2004). In the case of *D. sellowiana* in the SMOR, the forest was in different stages of regeneration, as mentioned in the previous paragraph, so the structure was dynamic in four populations, and normal in three, which is consistent with the results observed in other populations of the same species in different provinces in Brazil and Colombia, where individuals of lower height were the most abundant in places with different type of anthropic disturbance (Biondi *et al.*, 2009; Alfonso-Moreno *et al.*, 2011; Gasper *et al.*, 2011; Weber *et al.*, 2015). Predominance of seedlings, young, mature or old individuals is a product of the interaction between multiple environmental factors and/or random events at the local level (selective extraction of plants, grazing, opening of roads, natural or induced fires, landslides, dry years, among others) in a given time and space (Marques and Krupek, 2014; Pérez-Paredes, Sánchez-González, and Tejero-Díez, 2014), which is why it is common to find tree fern populations with different size or age structures. For example, Marques and Krupek (2014) recorded regressive populations (in which the number of mature individuals was larger than the number of young ones) of *D. sellowiana* in a fragment of forest in good condition (high humidity, low anthropogenic activity) in southern Brazil.

The predominance of individuals of less than 1.2 m in four of the seven populations analyzed in the present study could be considered as a criterion to define stability or instability of *D. sellowiana* populations (Agurauja, Moora, and Zobel, 2004; Odland, 2007; Agurauja *et al.*, 2008), or as an indicator that environmental conditions are suitable for spore germination, gametophyte formation, sexual reproduction, and seedling development (Large and Braggins, 2004; Sharpe and Mehltreter, 2010; Hernán-Cassini, 2013).

Under controlled conditions, the spore germination rate of *D. sellowiana* is high (88%) and the spore viability is sufficient to generate a spore bank in the forest (Fiilippini, Duz, and Randi, 1999; Schmitz *et al.*, 2006). Consequently, it is important to identify the environmental factors most correlated with survival in other perhaps more critical stages of its life cycle, such as

recruitment and subsequent development of seedlings (Sharpe and Mehltreter, 2010), in order to make suitable proposals for management and conservation of the species (Bernabe, Williams-Linera, and Palacios-Rios, 1999). The predominance of intermediate-sized individuals in the populations classified as normal or stable (Agua Blanca, Naupan, and Tlanchinol) could be related to moderate disturbance events, either natural or caused by human activities (Pérez-Paredes, Sánchez-González, and Tejero-Díez, 2014) such as “selective extraction” of small individuals or parts of robust individuals (as in the Naupan and Tlanchinol localities), and/or low availability of micro-environments for the establishment of seedlings because of factors such as landslides or trail-opening (Bernabe, Williams-Linera, and Palacios-Rios, 1999; Sharpe and Mehltreter, 2010).

Fertility in fern populations tends to vary spatially and temporally (Sharpe and Mehltreter, 2010)—in the present study, differences were found in fertility percentages between populations, especially in the classes under 0.58 m, so it is possible either that physiological development at this height is not enough for the plants to produce spores or that local environmental conditions are not favorable for fertility (Bernabe, Williams-Linera, and Palacios-Rios, 1999; Sharpe and Mehltreter, 2010). According to Magna-Silva *et al.* (2017), it is common in tree ferns to find that small-sized, fertile individuals actually originate from mature plants by vegetative reproduction. Field observations confirm this claim—some individuals of *D. sellowiana* were growing as aerial stems from recumbent and/or partially buried mature individuals.

Neumann, Schneider, and Schmitt (2014) suggest that *Cyathea corcovadensis* (Raddi) C. Christensen has the potential to increase its population density when the proportion of fertile individuals is over 60%. Assuming a similar reproductive behavior in other tree fern species, the mean fertility percentage in *D. sellowiana* at a regional level (SMOR) was high (67%). However, at the local level, the populations of Tizapan and Xocoyolo showed lower fertility percentages, 46.7 and 30.8%, respectively, so they could be considered as declining according to Neumann, Schneider, and Schmitt’s proposal (2014). In two *D. sellowiana* populations from southern Brazil, “low” fertility values were also recorded; 52%, and 38% (Schmitt, Schneider, and Windisch, 2009; Marques and Krupek, 2014, respectively), and, in a population in Colombia, only the individuals over 4 m in height (44.4%) were fertile (Ramírez-Valencia, Sanín, and Álvarez-Mejía, 2009). However, it would be necessary to carry out specific studies of the relationship between fertility and population density to determine whether the proposal of Neumann, Schneider, and Schmitt (2014) is accurate.

Intermediate and high basal area values (indirect measure of biomass) of the *D. sellowiana* populations occurred at elevations above 1,450 m, which is consistent with their climate and habitat preferences, given that their distribution is restricted to the MCF (Mickel and Smith, 2004), unlike other tree fern species such as *Alsophila firma* which tends to be successful at elevations lower than 1,470 m in the ecotone between the MCF and the sub-evergreen tropical forest in the states of Hidalgo and Puebla, in the SMOR,

Mexico (Eleuterio and Pérez-Salicrup, 2009; Pérez-Paredes, Sánchez-González, and Tejero-Díez, 2014). Elevation and latitude are environmental values that do not have a direct effect on plants—in contrast, temperature and humidity, which change along latitudinal and elevational gradients, do have a direct effect on the distribution of species (Bystriakova, Schneider, and Coomes, 2011; De Frenne *et al.*, 2013). The populations of *D. sellowiana* analyzed here are found at the northern limit of the neotropical distribution of the species—there are no records of tree ferns in the MCF of the SMOR in more northern latitudes, such as in the El Cielo Biosphere Reserve in Tamaulipas. It is probable that climate factors that vary negatively with latitude and elevation, such as temperature and precipitation (Jardel-Pelaez *et al.*, 2014), interfere with the abundance and distribution of tree fern species (Bystriakova, Schneider, and Coomes, 2011; De Frenne *et al.*, 2013; Sosa *et al.*, 2016).

The montane cloud forest is considered a highly vulnerable ecosystem on a worldwide level, due to its high degree of fragmentation and sparse occurrence. In Mexico, the main problem faced by this ecosystem is related to changes in land use to crop and livestock farming (Gual-Díaz and Rendón-Correa, 2014). In this scenario, it is increasingly important to study the distribution and structure of plant populations that are considered representative of the “typical” environmental conditions for this vegetation type. In the particular case of *D. sellowiana*, it is important to analyze the current state of its populations, because it is highly linked to its habitat conditions, which means that it could be used in restoration programs as well as for monitoring changes in forests over time.

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APPENDIX. Variables considered in the characterization of habitat. P: Sampling sites; D: Density, BA: Basal area, DIST: Distance from human settlements (min), ELV: Elevation (m), SL: Slope (degrees), OR: Orientation (degrees), GVC: Ground vegetation cover (%), CC: Canopy cover (%), RC: Rock cover (%), T: trails, UR: Unpaved roads, H: Highways, LS: Landslides (m²), EXT: Extraction/clearing.

P	D	BA	DIST	ELV	SL	OR	GVC	CC	RC	T	UR	H	LS	EXT
1	16	15481	11	1458.9	40	NW	37.5	69.8	5	1	1	1	1	2
2	22	24907	13	1458.9	55	NW	37.5	74.0	5	1	1	1	1	2
3	29	26461	15	1458.9	45	NW	62.7	63.1	5	1	1	1	1	2
4	32	12884	13	1878.0	35	NE	62.5	71.4	5	1	1	1	2	1
5	17	8639	13	1878.0	20	NE	37.5	77.1	5	1	1	1	2	1
6	43	4513	13	1878.0	33	NE	87.5	71.4	5	1	1	1	1	1
7	12	6481	16	1732.2	60	NW	17.5	73.0	17.5	1	1	1	1	2
8	12	4604	19	1732.2	35	NW	17.5	78.2	5	2	0	1	1	4
9	7	4476	22	1732.2	40	NW	62.5	78.2	5	1	0	1	0	5
10	35	19724	10	2191.9	40	NE	37.5	65.3	5	1	0	1	6	1
11	17	11007	10	2191.9	75	NE	62.5	78.2	17.5	1	1	0	8	1
12	18	12801	10	2191.9	70	NE	62.5	69.5	37.5	1	1	0	6	1
13	28	10071	20	1879.5	45	NE	62.5	74.8	5	1	1	0	0	1
14	29	7074	24	1879.5	40	NE	87.5	72.4	5	1	1	0	0	1
15	29	12292	30	1879.5	45	NE	87.5	77.38	17.5	1	1	0	0	1
16	14	6871	50	1917.7	65	NW	62.5	72.44	5	1	1	0	5	4
17	24	13151	45	1917.7	60	NW	87.5	52.16	5	3	1	1	120	4
18	21	9682	42	1917.7	45	NW	87.5	88.56	5	3	1	1	312	4
19	22	4657	18	1348	45	NW	62.5	75.04	5	1	1	0	1	6
20	15	3308	16	1348	55	NW	62.5	67.5	5	1	1	0	1	7
21	29	4962	14	1348	45	NW	87.5	77.12	5	1	1	0	1	6

Leaf Anatomy of Two Fern Species and a Hybrid of *Anemia* (Anemiaceae)

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ABSTRACT.—The present study describes the anatomy of *Anemia villosa*, *Anemia organensis*, and a hybrid of the two species (*Anemia villosa* × *Anemia organensis*). The aim of the present study was to answer the following question: can the *Anemia villosa* × *Anemia organensis* hybrid be distinguished from the parent plants based on its anatomical traits? The results showed that the two species and hybrid can be easily differentiated by their anatomical characteristics and that the hybrid anatomy shares features with both parents. In addition, the hybrid differed from the parents in two variables: leaf thickness and number of trichome cells.

KEY WORDS.—*Anemia organensis*, *Anemia villosa*, discriminant analysis, hybridization, stele.

Hybridization is common in plants, especially ferns (Barrington, 1989). *Anemia* is a fern genus of the family Anemiaceae with 115 species in the Neotropical region and 17 in Africa, Madagascar, Réunion, and southern India (Mickel, 2016). Many *Anemia* species are apparently of hybrid origin, and 35 hybrid combinations have been identified in the Neotropical region, with more likely to be recorded (Mickel, 1962; 1982; 2016; Pena, Schwartsburd, and Alves-Araújo, 2017; Rabelo and Schwartsburd, 2016). Brazil is the primary center of diversity of *Anemia* with 66 species, of which 39 are endemic (*Anemia* in Flora do Brasil 2020) and 19 hybrids recorded (Mickel, 2016; Pena, Schwartsburd, and Alves-Araújo, 2017; Rabelo and Schwartsburd, 2016; Santos and Araújo, 2007).

Anemia organensis Rosenst. is an endemic species of the Brazilian Atlantic Rain Forest (*Anemia* in Flora do Brasil, 2020) and only one hybrid with *Anemia villosa* Humb. & Bonpl. ex Willd. has been recorded for the municipality of Santa Maria Magdalena, Rio de Janeiro State, Brazil (Mickel, 1962; 2016). In addition to the hybrid with *A. organensis*, hybrids of *A. villosa* have been recorded with *A. raddiana* and *A. imbricata*, all in Brazil (Mickel, 2016).

Morphological data are frequently used to verify the occurrence of hybrids in ferns (e. g., Kott and Britton, 1982; Barrington, 1986; Paris and Windham, 1988; Haines, 2003), and other groups (Fant and Preston, 2004). Anatomical features

can also be useful in hybrid identification (e.g., Webb and Carlquist, 1964; Brandham and Cutler, 1978; Baas, 1978). According to Baas (1978), anatomy deserves greater attention and could be used to verify the occurrence of hybrids. Hybridization research on ferns typically considers only morphology or cytology (e.g., Kott and Britton, 1982; Barrington, 1986; Paris and Windham, 1988) and we found no studies in the literature on the anatomy of fern hybrids.

Some studies involve hybrids that are artificially produced in laboratories and compare their anatomy with naturally occurring specimens. This kind of research requires a range of data to accurately identify hybrids (Judd *et al.*, 1999). Multivariate exploratory techniques are useful in distinguishing hybrids and principal component analysis (PCA) and discriminant factor analysis (DFA) have been used by a number of researchers (Thébaud and Abbot, 1995; Gurgerli, 1997; Levin, 1999; Albaladejo, Aparicio, and Silvestre, 2004).

The present study describes the anatomy of *Anemia villosa*, *Anemia organensis*, and a hybrid of the two species (*Anemia villosa* x *Anemia organensis*). The aim is to answer the following question: can the hybrid (*Anemia villosa* x *Anemia organensis*) be distinguished from the parent plants based on anatomical variables? And if so, what anatomical characters are involved?

MATERIAL AND METHODS

Leaf samples of *Anemia villosa*, *Anemia organensis* and the *Anemia villosa* x *Anemia organensis* hybrid were collected along the "Pedra Dubois" trail, in the municipality of Santa Maria Madalena (21°45'-22°00'S and 41°41'-42°00'W), Rio de Janeiro state, Brazil.

Five individual parent plants and hybrids were randomly selected (N=15). Five fully developed leaves were collected from each and immediately fixed in 70% formalin-acetic acid-alcohol in the field (FAA) (Berlyn and Miksche, 1976). Next, the material (0.5 cm² of each leaf) was gradually dehydrated in ethanol and embedded in glycol methacrylate resin (Feder and O'Brien, 1968). Sections (5 µm) were cut using a rotary microtome and subsequently stained with 0.1% Toluidine blue O (O'Brien and McCully, 1965).

Stomatal density was estimated in dissociated material using Jeffrey and Franklyn's reagent (Jensen, 1962).

Anatomical descriptions and measurements were performed using an Olympus BX50 light microscope and image analysis software (Image-Pro Plus, version 3). The images were obtained with a Cool SNAP-Pro digital camera attached to the microscope.

For scanning electron microscopy, leaf fragments were fixed in 2.5% glutaraldehyde, 4% formaldehyde in 0.05 M cacodylate buffer. After dehydration in acetone, the samples were placed in a critical point Bal-Tec Critical Point Dryer CPD 030. The fragments were then affixed with carbon tape on their own supports and covered with a thin layer of 20 nm gold (Bal-Tec Sputer Coater SCD 050). The samples were then observed and electro-

micrographed under the scanning electron microscope ZEISS DSEM 962 and EVO 40 at a voltage of 25KV.

The spores were collected in the field, packed in envelopes and mounted on slides with glycerin, according to Large and Braggins (1990).

The following leaf anatomical parameters were measured for each population: thickness of mesophyll, palisade and spongy parenchyma, adaxial and abaxial epidermis, and leaf (vein and non-vein regions). The sample size established was 25 fields for each measurement ($N=25$), from which the means and standard deviations were calculated. The results of the measurements were used in a simple correlation between anatomical traits, and the non-anatomical factors were assessed. The significance of differences between the populations studied was established by multivariate analysis of variance (ANOVA). Principal components analysis (PCA) was used to group the populations and identify the components with the highest variance. Discriminant Function Analysis (DFA) was applied to verify the hypothesis that the study sample consisted of discrete groups that are morphologically different from each other (Schlichting, 1986; Zar, 1996). Overall differences between the groups are expressed as the Mahalanobis distance. The statistical analyses were performed using Statistica v. 6.0 software (Statsoft).

RESULTS

The laminae in the parent plants and hybrid are 1-pinnate-pinnatifid in *A. villosa*, 1-pinnate in *A. organensis*, and a shallowly pinnatifid laminae in the hybrid. The spores of *Anemia villosa* and *Anemia organensis* are trilete, averaging 84.18 μm and 92.02 μm long, respectively. The hybrid spores are deformed, with an average length of 81.73.

The petiole (Fig. 1) of the parent species and hybrid exhibit a uniseriate epidermis with lignified walls. The stele is "V" shaped in *A. villosa* (Fig. 1 A) and "C" shaped in *A. organensis* and the hybrid (Fig. 1 B and C).

Both species and the hybrid displayed hypostomatic leaves (Fig. 2 B, D, and E). A single-layer epidermis was observed, with sinuous anticlinal cell walls (Fig. 2 B, D, and E). Stigmata are present on the epidermis cells above the veins (Fig. 3 G, H, and I). In the sections, the external periclinal walls of the epidermal cells are convex. Multicellular trichomes are present in both species and the hybrid.

The mesophyll in both species and the hybrid contains 3-4 layers of branched parenchyma (Fig. 3). The first layer below the adaxial epidermis is more compact. In *A. organensis* (Fig. 3 L) and the hybrid (Fig. 3 K), the first and second layers below the adaxial epidermis display elongated cells (Fig. 3 K and L).

The leaf margin differs between the studied species and is straighter in *A. villosa* (Fig. 3 J), but angled toward the abaxial surface in the hybrid and *A. organensis*. In the hybrid, the leaf margin has a vein consisting only of xylem cells (Fig. 3 K).

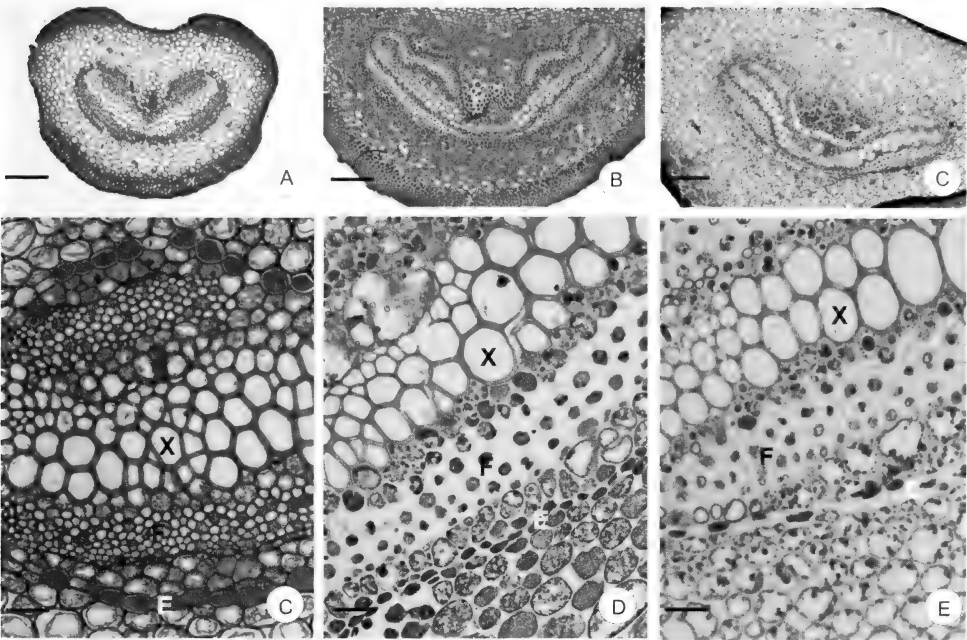


FIG. 1. Petiole, transverse section. (A, D) *Anemia villosa* (B, E), hybrid, and (C, F) *Anemia organensis* Bars= 350µm (A, B, and C) and 100 µm (D, E and F). E=endodermis, F= phloem and X= xylem.

Table 1 shows the ANOVA results of the groups for the quantitative variables. The parent groups differed for 10 of the 11 variables studied. The hybrid differed from the parents in two variables: leaf thickness and number of trichome cells. The hybrid is similar to *A. organensis* in five variables (adaxial surface epidermal thickness, stomatal length, leaf thickness in the non-vein region, mesophyll thickness, and trichome density on the adaxial surface) and to *A. villosa* in four variables (abaxial surface epidermal thickness, stomatal density, trichome density on the abaxial surface, and spore length)

Factor loadings and eigenvalues for the first two components (PCs1 and 2) extracted in PCA are shown in Table 2. These accounted for 62% of the total variance. Anatomical traits such as leaf thickness, mesophyll thickness, and trichome density on the adaxial surface showed the highest (either positive or negative) correlations with PC1, and trichome density on the abaxial surface and spore length exhibited the highest correlations with PC2. The scatterplot (Fig. 4) for these two components indicated three distinct groups.

Factor structure for the 11 variables and eigenvalues for the first two factors (DF1 and 2) extracted in DFA are shown in table 2. DF1 was positively correlated with leaf thickness and negatively with stomatal density, whereas DF2 was positively correlated with the stomatal density. The DFA classification was 100% for individuals from groups I, II, and III.

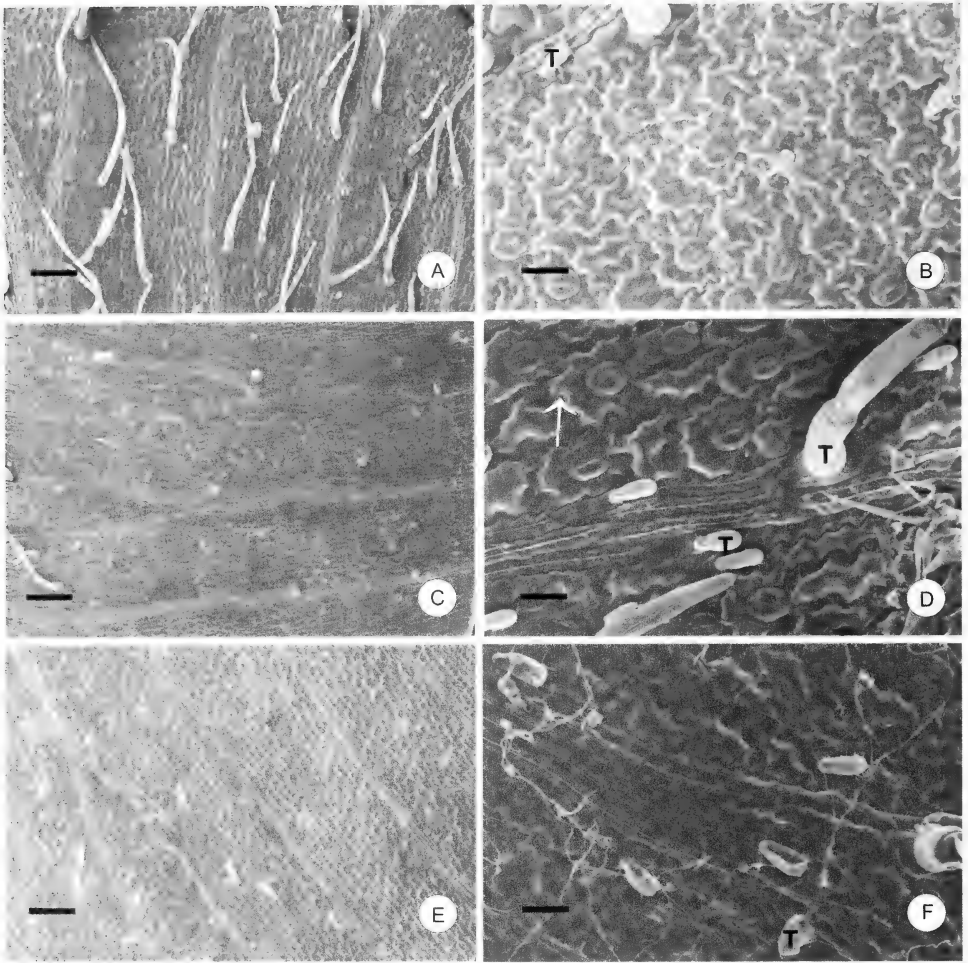


FIG. 2. Epidermis, scanning electron micrographs. *Anemia villosa* on the adaxial (A) and abaxial surface (B). Hybrid, on the adaxial (C) and abaxial surface (D). *Anemia organensis* on the adaxial (E) and abaxial surface (F). Bars= 200 μ m (A, C, E); 20 μ m (B, D, and F). T= trichomes, \rightarrow sinuous anticlinal wall.

DISCUSSION

The results showed differences between the hybrid and parent plants in terms of quantitative and qualitative anatomical characters. Mickel (1962) described the *Anemia organensis* \times *Anemia villosa* hybrid considering only the shallowly pinnatifid laminae and aborted spores. These morphological characteristics are intermediate between the pinnate-pinnatifid laminae in *A. villosa* and 1-pinnate laminae in *S. organensis* (Mickel, 2016). Moreover, the hybrid spores were deformed, whereas *A. villosa* and *A. organensis* displayed trilete spores.

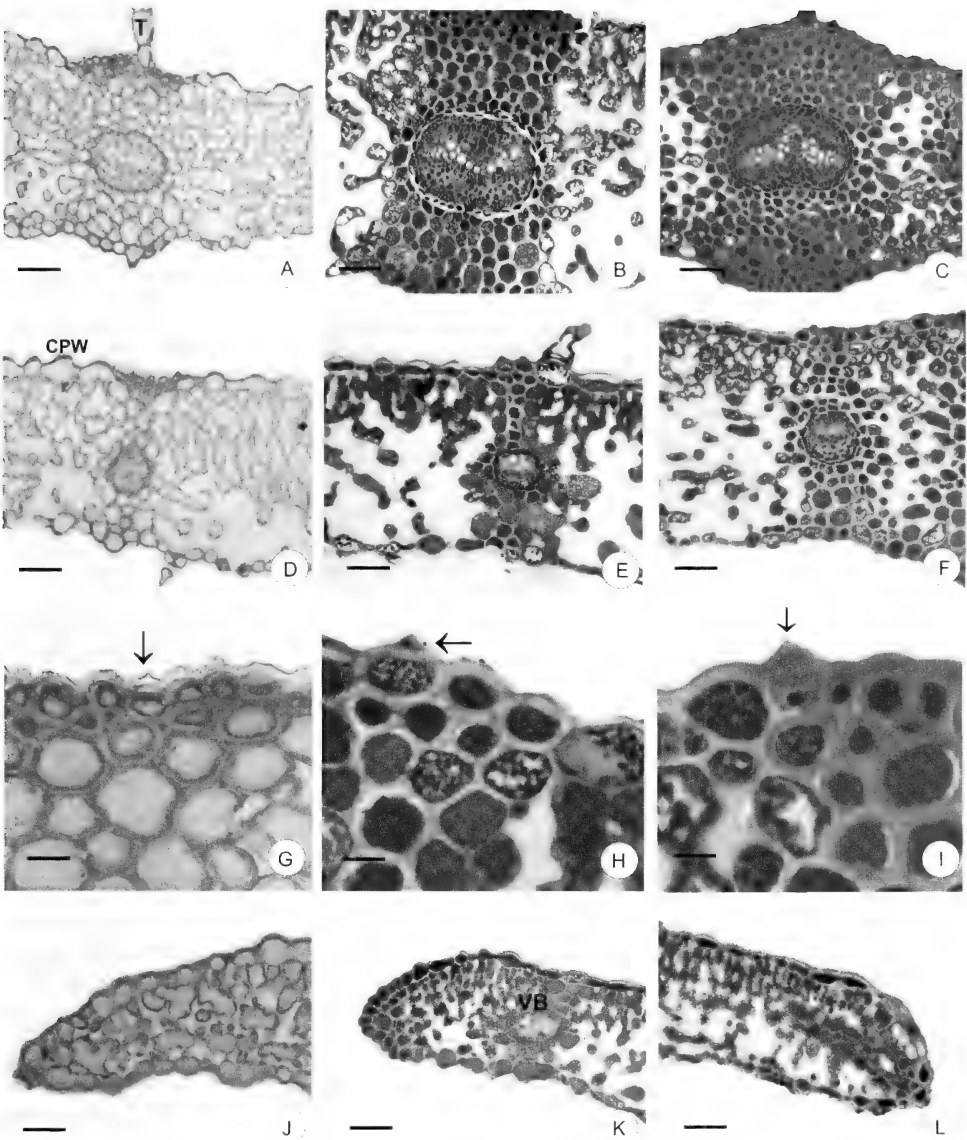


FIG. 3. Main vein region, secondary veins, stigmata and leaf margin, transverse sections (A, D, G, and J) *Anemia villosa*, (B, E, H, and K), hybrid (C, F, I, and L), and *Anemia organensis* Link. Bars= 100 μ m (A, B, C, D, E, and F), 25 μ m (G, H, and I) and 50 μ m (J, K, and L). → stigmata, VB= vascular bundle, T= trichome, CPW= convex periclinal wall.

Baas (1978) studied hybridization in *Ilex* species and identified four possible hybrid anatomies: 1) intermediate between the parent species; 2) inheriting the trait of one of the parents 3) lacking the trait of one of the parents, or 4) showing a 'new' character absent from both parents. In the present study, the

TABLE 1. Variables of *Anemia organensis*, *Anemia villosa* and the hybrid (*A. organensis* x *A. villosa*). Means±SD (standard deviation) and F values (ANOVA, *P<0,05). Means followed by different letters differ at P<0.05 (Tukey’s HSD test)

	<i>A.organensis</i>	<i>A. villosa</i>	hybrid	F
Epidermal thickness - adaxial surface (µm)	20.02±2.89a	23,06±5.55b	20.51±3.52a	14.71*
Epidermal thickness - abaxial surface (µm)	21.77±3.74a	25.82±7.09b	25.63±3.33b	21.70*
Stomatal length (µm)	42.61±4.26a	52.67±4.34b	41.49±2.93a	191.81*
Stomatal density (mm)	42.80±6.47a	47.83±3.56b	47.42±2.97b	12.27*
Leaf thickness in vein region (µm)	314.83±44.47a	219.05±29.22b	326.64±59.49a	132.18*
Leaf thickness non-vein region (µm)	506.72±51.38a	308.08±25.44b	357.52±42.33c	557.38*
Mesophyll thickness (µm)	279.79±44.91a	16.57±15.73b	287.36±57.37a	216.55*
Trichome density - adaxial surface (mm)	3.27±1.13a	7.23±1.68b	3±1.06a	132.42*
Trichome density - abaxial surface (mm)	15.17±3.45a	15.59±3.45a	26.90±4.35b	115.66*
Number of trichome cells	8.14±1.19a	6.80±0.94b	5.60±1.40c	30.17*
Spore length (m)	92.02±5.49a	84.18±6.23b	81.73±10.20b	55.39*

hybrid has a vein on the leaf margin consisting only of xylem cells not present in the parent plants. With respect to quantitative variables, the hybrid differed from the parent plants in 2 of the 11 variables, with 5 variables similar to *A. organensis* and 4 to *A. villosa*. Thus, in relation to quantitative variables, the hybrid exhibits traits from both parent plants.

Webb and Carlquist (1964) investigated *Salvia* species and found that trichome density on the abaxial and adaxial leaf surfaces was useful in discriminating hybrids from parents. In the present study, trichome density was higher on the adaxial surface in *A. villosa* and on the abaxial surface in the hybrid.

There were significant differences in stomatal length between the taxa studied. The stomata of *A. organensis* and the hybrid showed similar values, but were larger in *A. villosa*. Similar data were obtained by Brandham and Cutler (1978) in a study with *Aloe* species.

Multivariate exploratory techniques (PCA and DFA) were important in distinguishing between the hybrid and parent plants. The results showed

TABLE 2. Factor loadings in PCA and factor structure coefficients in DFA for the variables of *Anemia organensis*, *Anemia villosa* and the hybrid (*A. organensis* x *A. villosa*).

Variables	PC1	PC2	DF1	DF2
Stomatal density	0.272	0.393	-0.406	0.526
Thickness	-0.903	0.120	0.917	-0.072
Mesophyll thickness	-0.907	0.101	0.055	-0.490
Epidermal thickness - adaxial surface	-0.219	0.164	-0.109	-0.038
Trichome density - adaxial surface	0.729	-0.202	-0.021	0.468
Trichome density - abaxial surface	-0.361	0.785	-0.316	-0.432
Spore length	-0.205	-0.693	0.191	-0.084

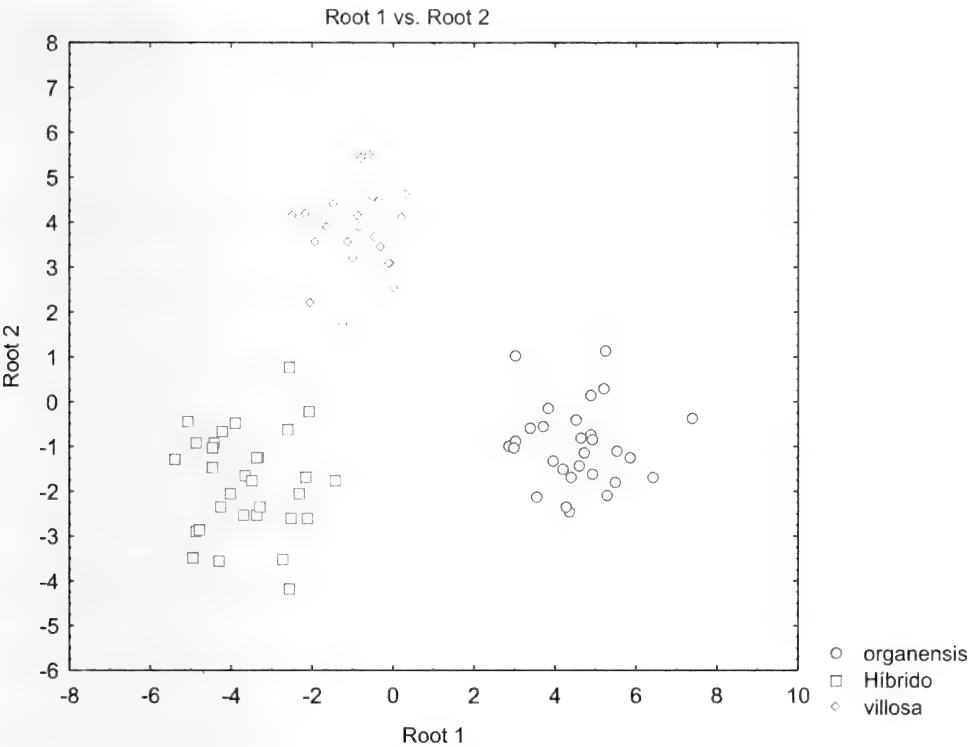


FIG. 4. Discriminant analysis of *Anemia villosa*, *Anemia organensis*, and hybrid.

100% separation between the groups. Thébaud and Abbot (1995), Gurgeli (1997), Levin (1999), and Albaladejo and Silvestre (2004) used the same techniques to separate hybrids, and the last authors also obtained 100% separation.

In conclusion, hybrids can be distinguished from parent plants by their morphological and anatomical characteristics. Hybrid morphology is intermediate in relation to parent plants, whereas the anatomy of hybrids shares features with parent plants and exhibits unique characteristics (leaf thickness and number of trichome cells). The hybrid is similar to *A. organensis* in five variables (adaxial surface epidermal thickness, stomatal length, leaf thickness in the non-vein region, mesophyll thickness, and trichome density on the adaxial surface) and to *A. villosa* in four variables (abaxial surface epidermal thickness, stomatal density, trichome density on the abaxial surface, and spore length).

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Taxonomic and Nomenclatural Updates to the Fern and Lycophyte Flora of the Hawaiian Islands

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ABSTRACT.—We present here updates to the known species of ferns and lycophytes in the Hawaiian Islands since the publication of Palmer (2003) and the overview and updates of Vernon and Ranker (2013).

Palmer (2003) represented the first thorough and concise presentation of the diversity of ferns and lycophytes on the Hawaiian Islands. Vernon and Ranker (2013) provided updates to the status of known species up to that time, including nomenclatural changes and the addition of newly named species and new records of naturalized species. Since that last update one additional new species was described (Wood and Wagner, 2017) and several additional naturalized, non-native species have been documented (see references below). Thus, we present here an updated checklist of the native and naturalized ferns and lycophytes of the Hawaiian Islands based on the references cited below. The checklist follows the familial and generic classification system recommended by PPG I (2016). The checklist also includes known and putative hybrids and recognized forms, not included in Vernon and Ranker (2013).

A spreadsheet version of this checklist may be downloaded at: <http://www.botany.hawaii.edu/people/tom-ranker/>

Abbreviations: ^E endemic; ^I indigenous; ^{NZ} naturalized; (ex)=extinct; USFWS=U. S. Fish & Wildlife Service; N=Ni'ihau; Le=Lehua; K=Kaua'i; O=O'ahu; Mo=Moloka'i; L=Lāna'i; Ma=Maui; Ka=Kaho'olawe; H=Hawai'i

* Synonyms listed are those that represent updates subsequent to Palmer (2003)

LYCOPHYTES

1. *Lycopodiaceae* P.Beauv. ex Mirb.

Huperzia Bernh.

^E *Huperzia erosa* Beitel & W.H.Wagner

Distribution: K/O/Mo/L/Ma/H

^I *Huperzia erubescens* (Brack.) Holub

Distribution: K/O/Mo/Ma/H

^I *Huperzia haleakalae* (Brack.) Holub

Distribution: Ma(ex)

^I *Huperzia serrata* (Thunb. ex Murray) Trevis.

Distribution: K/O/Mo/L/H

- ^E *Huperzia subintegra* (Hillebr.) Beitel & W.H.Wagner

Distribution: K/O/Mo/Ma

- ^E *Huperzia* × *carlquistii* Beitel & W.H.Wagner (*Huperzia erubescens* × *H. subintegra*)

Distribution: O/Ma

- ^E *Huperzia erubescens* (Brack.) Holub × *H. haleakalae* (Brack.) Holub

Distribution: K/Ma

- ^E *Huperzia* × *gillettii* Beitel & W.H.Wagner (*Huperzia serrata* × *H. subintegra*)

Distribution: K/O/Mo/Ma/H

- ^E *Huperzia* × *medeirosii* Beitel & W.H.Wagner (*Huperzia haleakalae* × *H. subintegra*)

Distribution: Ma

- ^E *Huperzia sulcinervia* (Spring) Trevis. (*Huperzia erubescens* × *H. serrata*)

Distribution: O/L/H

Lycopodium L.

- ^I *Lycopodium venustulum* Gaudich. var. *venustulum*

Distribution: K/O/Mo/L/Ma/H

- ^E *Lycopodium venustulum* Gaudich. var. *verticale* W.H.Wagner

Distribution: H

Palhinhaea Franco & Carv.

- ^I *Palhinhaea cernua* (L.) Franco & Vasc.

Syn. *Lycopodiella cernua* (L.) Pic.Serm.

Distribution: K/O/Mo/L/Ma/H

Phlegmariurus (Herter) Holub

- ^I *Phlegmariurus filiformis* (Sw.) W.H.Wagner

Syn. *Huperzia filiformis* (Sw.) Holub

Distribution: K/O/Mo/L/Ma/H

- ^E *Phlegmariurus mannii* (Hillebr.) W.H.Wagner

Syn. *Huperzia mannii* (Hillebr.) Kartesz & Gandhi

Distribution: K(ex)/Ma/H

USFWS category: Endangered

- ^E *Phlegmariurus nutans* (Brack.) W.H.Wagner

Syn. *Huperzia nutans* (Brack.) Rothm.

Distribution: K(ex)/O

USFWS category: Endangered

- ^I *Phlegmariurus phyllanthus* (Hook. & Arn.) R.D.Dixit

Syn. *Huperzia phyllantha* (Hook. & Arn.) Holub

Distribution: K/O/Mo/L/Ma/H

- ^E *Phlegmariurus stemmermanniae* A.C.Medeiros & W.H.Wagner

Syn. *Huperzia stemmermanniae* (A.C.Medeiros & W.H.Wagner)

Kartesz

Distribution: Ma/H

USFWS category: Endangered

- ^E *Phlegmariurus* × *koolauensis* W.H.Wagner (*Phlegmariurus nutans* × *P. phyllanthus*)
Distribution: O
2. **Isoëtaceae** Reichenb.
Isoëtes L.
^E *Isoëtes hawaiiensis* W.C.Taylor & W.H.Wagner
Distribution: Ma/H
3. **Selaginellaceae** Willk.
Selaginella P. Beauv.
^I *Selaginella arbuscula* (Kaulf.) Spring
Distribution: K/O/Mo/L/Ma/H
Note: *Selaginella arbuscula* was previously considered an endemic species. This species has a greater distribution, occurring in the Society Islands, Ualan, Santa Cruz Island (Vanikoro), and the Marquesas Islands (Hassler and Swale, 2019).
^E *Selaginella deflexa* Brack.
Distribution: K/O/Mo/Ma/H
^{NZ} *Selaginella kraussiana* (Kunze) A.Braun
Distribution: O/Ma/H
^{NZ} *Selaginella laxa* Spring
Distribution: K
New record based on specimens: *Palmer 582 & Palmer 583* (HAW)
^{NZ} *Selaginella stellata* Spring
Distribution: H
^{NZ} *Selaginella umbrosa* Lem. ex Hieron.
Distribution: H

FERNS

1. **Ophioglossaceae** Martinov
Ophioderma (Blume) Endl.
^I *Ophioderma pendulum* (L.) C.Presl
subsp. *falcatum* (C.Presl) R.T.Clausen
Distribution: K/O/Mo/L/Ma/H
subsp. *pendulum*
Distribution: O/Mo/Ma/H
Ophioglossum L.
^I *Ophioglossum nudicaule* L.f.
Distribution: K/O/H
^I *Ophioglossum petiolatum* Hook.
Distribution: K/O/Mo/L/Ma/H
^I *Ophioglossum polyphyllum* A.Braun
Distribution: K/O/Mo/L(ex)/Ka/Ma/H
Sceptridium Lyon
^E *Sceptridium subbifoliatum* (Brack.) Lyon

Distribution: K(ex)/O(ex)/Mo(ex)/L(ex)/Ma(ex)/H(ex)

2. **Psilotaceae** J.W.Griff. & Henfr.

Psilotum Sw.

¹ **Psilotum complanatum** Sw.

Distribution: K/O/Mo/L/Ma/H

¹ **Psilotum nudum** (L.) P. Beauv.

Distribution: N/K/O/Mo/L/Ka/Ma/H

¹ **Psilotum** × **intermedium** W.H.Wagner (*Psilotum complanatum* × *P. nudum*)

Distribution: K/O/L/Ma

3. **Marattiaceae** Kaulf.

Angiopteris Hoffm.

^{NZ} **Angiopteris evecta** (G.Forst.) Hoffm.

Distribution: K/O/Mo/L/Ma/H

Marattia Sw.

^E **Marattia douglasii** (C.Presl) Baker

Distribution: K/O/Mo/L/Ma/H

4. **Hymenophyllaceae** Mart.

Callistopteris Copel.

^E **Callistopteris baldwinii** (D.C.Eaton) Copel.

Distribution: K/O/Mo/L/Ma/H

Crepidomanes C. Presl

^E **Crepidomanes draytonianum** (Brack.) Ebihara & K.Iwats.

Syn. *Vandenboschia draytoniana* (Brack.) Copel.

Distribution: K/O/Mo/L/Ma/H

¹ **Crepidomanes minutum** (Blume) K.Iwats.

Syn. *Gonocormus minutus* (Blume) Bosch; *Crepidomanes proliferum* (Blume) Bostock; *Gonocormus prolifer* (Blume) Prantl

Distribution: K/O/Mo/L/Ma/H

Hymenophyllum Sm.

^E **Hymenophyllum lanceolatum** Hook. & Arn.

Syn. *Sphaerocionium lanceolatum* (Hook. & Arn.) Copel.

Distribution: K/O/Mo/L/Ma/H

^E **Hymenophyllum obtusum** Hook. & Arn.

Syn. *Sphaerocionium obtusum* (Hook. & Arn.) Copel.

Distribution: O/Mo/L/Ma/H

^E **Hymenophyllum recurvum** Gaudich.

Syn. *Mecodium recurvum* (Gaudich.) Copel.

Distribution: K/O/Mo/L/Ma/H

Vandenboschia Copel.

^E **Vandenboschia cyrtotheca** (Hillebr.) Copel.

Distribution: K/O/Mo/L/Ma/H

^E **Vandenboschia davallioides** (Gaudich.) Copel.

Distribution: K/O/Mo/L/Ma/H

^E **Vandenboschia tubiflora** F.S.Wagner

Distribution: K

5. **Gleicheniaceae** C. Presl**Dicranopteris** Bernh.^I ***Dicranopteris linearis*** (Burm.f.) Underw.

Distribution: K/O/Mo/L/Ma/H

Includes: *D. linearis* (Burm. f.) Underw. f. *linearis* and *D. linearis* (Burm. f.) Underw. f. *emarginata* (Brack.) W.H.Wagner**Diplopterygium** (Diels) Nakai^E ***Diplopterygium pinnatum*** (Kunze) Nakai

Distribution: K/O/Mo/L/Ma/H

Sticherus C. Presl^E ***Sticherus owbyhensis*** (Hook.) Ching

Distribution: K/O/Mo/L/Ma/H

6. **Lygodiaceae** M.Roem.**Lygodium** Sw.^{NZ} ***Lygodium japonicum*** (Thunb.) Sw.

Distribution: O/H

7. **Schizaeaceae** Kaulf.**Schizaea** Sm.^E ***Schizaea robusta*** Baker

Distribution: K/O/Mo/L/Ma/H

8. **Marsileaceae** Mirb.**Marsilea** L.^{NZ} ***Marsilea minuta*** L.Syn. *Marsilea crenata* C.Presl

Distribution: O

^E ***Marsilea villosa*** Kaulf.

Distribution: N/O/Mo

USFWS category: Endangered

9. **Salviniaceae** Martinov**Azolla** Lam.^{NZ} ***Azolla filiculoides*** Lam.

Distribution: K/O/Mo/L/Ma/H

Salvinia Seg.^{NZ} ***Salvinia molesta*** D.S.Mitch.

Distribution: O/H

10. **Cibotiaceae** Korall**Cibotium** Kaulf.^E ***Cibotium chamissoi*** Kaulf.

Distribution: O/Mo/L/Ma/H

^E ***Cibotium glaucum*** (Sm.) Hook. & Arn.

Distribution: K/O/Mo/L/Ma/H

^E ***Cibotium menziesii*** Hook.

Distribution: K/O/Mo/L/Ma/H

^E ***Cibotium nealiae*** O.Deg.

Distribution: K

^E ***Cibotium* × *heleniae*** D.D.Palmer (*Cibotium chamissoi* × *C. menziesii*)

Distribution: O

11. **Cyatheaceae** Kaulf.

Sphaeropteris Bernh.

^{NZ} ***Sphaeropteris cooperi*** (Hook. ex F.Muell.) R.M.Tryon

Distribution: K/O/L/Ma/H

12. **Dicksoniaceae** M.R. Schomb.

Dicksonia L'Hér.

^{NZ} ***Dicksonia fibrosa*** Colenso

Distribution: H

New record (Lorence and Flynn, 2006)

13. **Lindsaeaceae** C. Presl ex M.R. Schomb.

Lindsaea Dryand. ex Sm.

^{NZ} ***Lindsaea ensifolia*** Sw.

Distribution: K/O/Mo/Ma/H

Lindsaea repens (Bory) Thwaites

^E var. ***macraeana*** (Hook. & Arn.) C.Chr.

Distribution: K/O/Mo/L/Ma/H

Odontosoria Fée

^I ***Odontosoria chinensis*** (L.) J.Sm.

Syn. *Sphenomeris chinensis* (L.) Maxon

Distribution: K/O/Mo/L/Ma/H

×***Lindsaeosoria*** W.H.Wagner

^E ×***Lindsaeosoria flynnii*** W.H.Wagner (*Lindsaea ensifolia* × *Odontosoria chinensis*)

Distribution: K/O

New island record for O (Frohlich and Lau, 2014)

14. **Dennstaedtiaceae** Lotsy

Hypolepis Bernh.

^E ***Hypolepis hawaiiensis*** Brownsey

var. ***hawaiiensis***

Distribution: K/O/Mo/L/Ma/H

var. ***mauiensis*** (Hillebr.) D.D.Palmer

Distribution: Ma

USFWS category: Endangered

Microlepia C. Presl

^I ***Microlepia speluncae*** (L.) T.Moore

Distribution: K/O/Mo/Ma/H

Microlepia strigosa (Thunb.) C.Presl

^E var. ***mauiensis*** (W.H.Wagner) D.D.Palmer

Distribution: O/Ma/H

USFWS category: Endangered

^I var. ***strigosa***

Distribution: K/O/Mo/L/Ma/H

^E ***Microlepia* ×*adulterina*** W.H.Wagner (*Microlepia speluncae* × *M. strigosa*)

Distribution: K/O/H

Pteridium Gled. ex Scop.

Pteridium aquilinum (L.) Kuhn

^E subsp. ***decompositum*** (Gaudich.) Lamoureux ex J.A.Thomson

Distribution: K/O/Mo/L/Ma/H

15. **Pteridaceae** E.D.M.Kirchn.

Adiantum L.

^{NZ} ***Adiantum 'Edwinii'***

Distribution: O/L/Ma

Palmer (2003) noted that this taxon may be a cultivar of either *A. raddianum* C.Presl or *A. concinnum* Willd. or a hybrid between them.

^I ***Adiantum capillus-veneris*** L.

Distribution: N/K/O/Mo/L/Ma/H

^{NZ} ***Adiantum hispidulum*** Sw.

Distribution: K/O/Mo/L/Ka/Ma/H

^{NZ} ***Adiantum macrophyllum*** Sw.

Distribution: O

New record (Lau and Frohlich, 2015)

^{NZ} ***Adiantum raddianum*** C.Presl

Distribution: K/O/Mo/L/Ma/H

^{NZ} ***Adiantum tenerum*** Sw.

Distribution: O/Ma

Ceratopteris Brongn.

^{NZ} ***Ceratopteris gaudichaudii*** Brongn. var. ***vulgaris*** Masuyama & Watano

Distribution: O

^{NZ} ***Ceratopteris thalictroides*** (L.) Brongn.

Distribution: K/O/H

Based on specimen annotations at BISH by S. Masuyama, November 2018

Cheilanthes Sw.

^{NZ} ***Cheilanthes viridis*** (Forssk.) Sw.

Distribution: K/O/L/Ma/H

Coniogramme Fée

^E ***Coniogramme pilosa*** (Brack.) Hieron.

Distribution: K/O/Mo/L/Ma/H

Doryopteris J.Sm.

^E ***Doryopteris angelica*** K.R.Wood & W.H.Wagner

Distribution: K

USFWS category: Endangered

^E ***Doryopteris decipiens*** (Hook.) J.Sm.

Distribution: N/K/O/Mo/L/Ka/Ma/H

^E ***Doryopteris decora*** Brack.

Distribution: K/O/Mo/L/Ka/Ma/H

^E ***Doryopteris subdecipiens*** W.H.Wagner (*Doryopteris decipiens* × *D. decora*)

Distribution: K/O/L/M/Ka/H

^E ***Doryopteris takeuchii*** (W.H.Wagner) W.H.Wagner

Distribution: O

USFWS category: Endangered

^E ***Doryopteris tryonii*** O.Deg. & I.Deg.

Distribution: K

Note: Possibly a variant of *D. decora*; needs further study.

See: Yesilyurt (2005)

Haplopteris C. Presl

^I ***Haplopteris elongata*** (Sw.) E.H.Crane

Distribution: K/O/Mo/L/Ma/H

Pellaea Link

^I ***Pellaea ternifolia*** (Cav.) Link

Distribution: K/O/Mo/L/Ma/H

Pityrogramma Link

^{NZ} ***Pityrogramma austroamericana*** Domin

Distribution: N/K/O/Mo/L/Ka/Ma/H

^{NZ} ***Pityrogramma calomelanos*** (L.) Link

Distribution: N/K/O/Mo/L/Ka/Ma/H

^E ***Pityrogramma* × *mckenneyi*** W.H.Wagner (*Pityrogramma austroamericana* × *P. calomelanos*)

Distribution: O

Pteris L.

^I ***Pteris cretica*** L.

Distribution: K/O/Mo/L/Ma/H

^E ***Pteris hillebrandii*** Copel.

Distribution: K/O/Mo/L/Ma/H

^E ***Pteris irregularis*** Kaulf.

Distribution: K/O/Mo/L/Ma/H

^E ***Pteris lidgatei*** (Baker) Christ

Distribution: O/Mo/Ma

USFWS category: Endangered

^I ***Pteris terminalis*** Wallich ex J.Agardh var. ***terminalis***

Syn. *Pteris excelsa* Gaudich.

Distribution: K/O/Mo/L/Ma/H

Note: See Ebihara et al. (2017) for explanation of synonym

^{NZ} ***Pteris tremula*** R.Br.

Distribution: Ma

New record (Oppenheimer, 2007)

^{NZ} ***Pteris vittata*** L.

Distribution: K/O/Mo/L/Ma/H

16. **Cystopteridaceae** (Payer) Shmakov

Cystopteris Bernh.

^E ***Cystopteris douglasii*** Hook.

Distribution: Ma/H

^E ***Cystopteris sandwicensis*** Brack.

Distribution: K/O/L/Ma

17. **Aspleniaceae** Newman***Asplenium* L.**^E ***Asplenium acuminatum* Hook. & Arn.**

Distribution: K/O/Mo/L/Ma/H

^I ***Asplenium adiantum-nigrum* L.**

Distribution: K/O/Mo/L/Ma/H

^I ***Asplenium aethiopicum* (Burm.f.) Bech.**

Distribution: K/O/Mo/L/Ma/H

^I ***Asplenium caudatum* G.Först.**Syn. *Asplenium horridum* Kaulf. var. *glabratum* (W.J.Rob.)D.D.Palmer; *Asplenium horridum* Kaulf. var. *horridum*

Distribution: K/O/Mo/L/Ma/H

Asplenium contiguum* Kaulf.**^I var. ***contiguum

Distribution: K/O/Mo/L/Ma/H

Note: Species listed as endemic in Palmer (2003), but reported also in Vanuatu by National Museum of Nature and Science (2008) and Ranker pers. obs. but needs critical evaluation.

^E var. ***hirtulum* C.Chr.**

Distribution: K/Ma

^E ***Asplenium dielerectum* Viane**Syn. *Diellia erecta* Brack.

Distribution: O/Mo/L(ex)/Ma/H

^E ***Asplenium dielfalcatum* Viane**Syn. *Diellia falcata* Brack.

Distribution: O

USFWS category: Endangered

^E ***Asplenium diellaciniatum* Viane**

Distribution: K

USFWS category: Endangered

See Lorence et al. (2013) for a discussion of this species.

^E ***Asplenium dielmannii* Viane**Syn. *Diellia mannii* (D.C.Eaton) W.J.Rob.

Distribution: K

USFWS category: Endangered

^E ***Asplenium dielpallidum* N.Snow**Syn. *Diellia pallida* W.H.Wagner

Distribution: K

USFWS category: Endangered

^E ***Asplenium haleakalense* W.H.Wagner**

Distribution: Ma/H

^E ***Asplenium hobdyi* W.H.Wagner**

Distribution: K/Mo/Ma/H

^I ***Asplenium insiticium* Brack.**

Distribution: K/O/Mo/L/Ma/H

^E ***Asplenium kaulfussii* Schltdl.**

Includes: *A. kaulfussii* Schltdl. f. *kaulfussii*, *A. kaulfussii* Schltdl. f. *gemniparum* (Hillebr.) D.D.Palmer, *A. kaulfussii* Schltdl. f. *bipinnatum* (Hillebr.) D.D.Palmer, and *A. kaulfussii* Schltdl. f. *dareoides* (Hillebr.) W.H.Wagner

Distribution: K/O/Mo/L/Ma/H

^E ***Asplenium leucostegioides* Baker**

Syn. *Diellia leucostegioides* (Baker) W.H.Wagner

Distribution: Ma(ex)

^I ***Asplenium lobulatum* Mett.**

Distribution: K/O/Mo/L/Ma/H

^I ***Asplenium macraei* Hook. & Grev.**

Status changed from Endemic to Indigenous; discovered in Marquesas Islands; see checklist: <https://naturalhistory2.si.edu/botany/marquesasflora/speciesdescr.cfm?genus=Asplenium&species=macraei>; also specimens from Savii, Samoa: Lorence et al. 10572 (PTBG, UC) and Flynn et al. 8626 (K, MBK, NY, PTBG, UC, US)

Distribution: K/O/Mo/L/Ma/H

^I ***Asplenium monanthes* L.**

Distribution: Ma/H

^I ***Asplenium nidus* L.**

Distribution: K/O/Mo/L/Ma/H

^I ***Asplenium normale* D.Don**

Distribution: K/O/Mo/L/Ma/H

***Asplenium peruvianum* Desv.**

^E var. *insulare* (C.V.Morton) D.D.Palmer

Distribution: Ma/H

USFWS category: Endangered

^I ***Asplenium polyodon* G.Forst.**

Distribution: K/O/Mo/L/Ma/H

^E ***Asplenium schizophyllum* C.Chr.**

Distribution: K/H

^E ***Asplenium sphenotomum* Hillebr.**

Distribution: K/O/Mo/L/Ma/H

***Asplenium trichomanes* L.**

^E subsp. *densum* (Brack.) W.H.Wagner

Distribution: Ma/H

^E ***Asplenium unisorum* (W.H.Wagner) Viane**

Syn. *Diellia unisora* W.H.Wagner

Distribution: O

USFWS category: Endangered

^E ***Asplenium* × *flagrum* W.H.Wagner & D.D.Palmer (*Asplenium hobdyi* × *A. normale*)**

Distribution: K/M

New island record for M (Oppenheimer, 2008)

^E ***Asplenium* × *joellauii* N.Snow (*Asplenium dielfalcatum* × *A. unisorum*)**

Distribution: O

New combination (Snow, 2011)

- ^E ***Asplenium* × *kokeense*** W.H.Wagner (*Asplenium aethiopicum* × *A. polyodon*)

Distribution: K/O

- ^E ***Asplenium* × *sphenocookii*** W.H.Wagner (*Asplenium polyodon* × *A. sphenotomum*)

Distribution: K

- ^E ***Asplenium* × *waikamoi*** W.H.Wagner (*Asplenium acuminatum* × *A. aethiopicum*)

Distribution: Ma

Hymenasplenium Hayata

- ¹ ***Hymenasplenium excisum*** (C.Presl) S.Linds.

Syn. *Asplenium excisum* C.Presl

Distribution: K/O/Mo/L/Ma/H

- ¹ ***Hymenasplenium unilaterale*** (Lam.) Hayata

Syn. *Asplenium unilaterale* Lam.

Distribution: K/O/Mo/L/Ma/H

18. **Thelypteridaceae** Ching ex Pic.Serm.

Amauropelta Kunze

- ^E ***Amauropelta globulifera*** (Brack.) Holttum

Syn. *Thelypteris globulifera* (Brack.) C.F.Reed

Distribution: K/O/Mo/L/Ma/H

Christella H.Lév.

- ^E ***Christella boydiae*** (D.C. Eaton) Holttum

Syn. *Cyclosorus boydiae* (D. C. Eaton) W.H.Wagner

Distribution: O/Ma

- ^E ***Christella cyatheoides*** (Kaulf.) Holttum

Syn. *Cyclosorus cyatheoides* (Kaulf.) Farw.

Distribution: K/O/Mo/L/Ma/H

- ^{NZ} ***Christella dentata*** (Forssk.) Brownsey & Jermy

Syn. *Cyclosorus dentatus* (Forssk.) Ching

Distribution: N/K/O/Mo/L/Ma/H

- ^{NZ} ***Christella parasitica*** (L.) H.Lév.

Syn. *Cyclosorus parasiticus* (L.) Farw.

Distribution: K/O/Mo/L/Ma/H

- ^E ***Christella wailele*** (Flynn) D.D.Palmer

Syn. *Cyclosorus wailele* (Flynn) W.H.Wagner

Distribution: K

- ^E ***Christella cyatheoides*** (Kaulf.) Holttum × ***C. dentata*** (Forssk.)

Brownsey & Jermy

Syn. *Cyclosorus* × *palmeri* (W.H.Wagner) W.H.Wagner; *Thelypteris*

× *palmeri* W.H.Wagner

Distribution: K/O

- ^E ***Christella dentata*** (Forssk.) Brownsey & Jermy × ***C. parasitica*** (L.)

H.Lév.

Syn. *Christella* \times *incesta* (W.H.Wagner) Nakaike & Kawabata; *Christella intermedius* (W.C.Shieh & J.L.Tsai) D.D.Palmer

Distribution: K/O/Ma

Cyclosorus Link

¹ **Cyclosorus interruptus** (Willd.) H.Itô

Distribution: K/O/Mo/L/Ma/H

Macrothelypteris (H. Itô) Ching

^{NZ} **Macrothelypteris torresiana** (Gaudich.) Ching

Distribution: K/O/Mo/Ma/H

New island record for Mo (Oppenheimer, 2016)

Pneumatopteris Nakai

^E **Pneumatopteris hudsoniana** (Brack.) Holttum

Syn. *Cyclosorus hudsonianus* (Brack.) Ching

Distribution: K/O/Mo/L/Ma/H

^E **Pneumatopteris pendens** D.D.Palmer

Syn. *Cyclosorus pendens* (D.D.Palmer) N.Snow

Distribution: K/O/Mo/Ma/H

^E **Pneumatopteris sandwicensis** (Brack.) Holttum

Syn. *Cyclosorus sandwicensis* (Brack.) Copel.

Distribution: K/O/Mo/L/Ma/H

Pseudophegopteris Ching

^E **Pseudophegopteris keraudreniana** (Gaudich.) Holttum

Distribution: K/O/Mo/L/Ma/H

19. **Blechnaceae** Newman

Blechnopsis C.Presl

^{NZ} **Blechnopsis orientalis** (L.) C.Presl

Syn. *Blechnum orientale* L.

Distribution: O

New State record (Lau and Frohlich, 2012)

Blechnum L.

^{NZ} **Blechnum appendiculatum** Willd.

Distribution: K/O/Mo/L/Ma/H

Doodia R. Br.

^E **Doodia kunthiana** Gaudich.

Distribution: K/O/Mo/L/Ma/H

^E **Doodia lyonii** O. Deg.

Distribution: K/O/Ma/H(ex)

Sadleria Kaulf.

^E **Sadleria cyatheoides** Kaulf.

Distribution: K/O/Mo/L/Ma/H

^E **Sadleria pallida** Hook. & Arn.

Distribution: K/O/Mo/L/Ma/H

^E **Sadleria souleyetiana** (Gaudich.) T.Moore

Distribution: K/O/Mo/L/Ma/H

^E **Sadleria squarrosa** (Gaudich.) T.Moore

Distribution: K/O/Mo/L/Ma/H

^E *Sadleria unisora* (Baker) W.J.Rob.

Distribution: K

^E *Sadleria wagneriana* D.D.Palmer & Flynn

Distribution: K

20. *Athyriaceae* Alston

Athyrium Roth

^E *Athyrium haleakalae* K.R.Wood & W.L.Wagner

Distribution: Ma

Newly described species (Wood and Wagner, 2017)

^E *Athyrium microphyllum* (Sm.) Alston

Distribution: K/O/Mo/L/Ma/H

Deparia Hook. & Grev.

^E *Deparia cataracticola* M.Kato

Distribution: K

^E *Deparia fenzliana* (Lueress.) M.Kato

Distribution: K/O/Mo/L/Ma/H

^E *Deparia kaalaana* (Copel.) M.Kato

Distribution: K(ex)/Ma(ex)/H(ex)

USFWS category: Endangered

^E *Deparia marginalis* (Hillebr.) M.Kato

Distribution: K/Mo/L/Ma/H

^{NZ} *Deparia petersenii* (Kunze) M.Kato subsp. *petersenii*

Distribution: K/O/Mo/L/Ma/H

^E *Deparia prolifera* (Kaulf.) Hook. & Grev.

Distribution: K/O/Mo/L/Ma/H

Diplazium Sw.

^E *Diplazium arnottii* Brack.

Distribution: K/O/Mo/L/Ma/H

^{NZ} *Diplazium esculentum* (Retz.) Sw.

Distribution: K/O/Mo/L/Ma/H

^E *Diplazium molokaiense* W.J.Rob.

Distribution: K(ex)/O(ex)/Mo(ex)/L(ex)/Ma

USFWS category: Endangered

^E *Diplazium sandwichianum* (C. Presl) Diels

Distribution: K/O/Mo/L/Ma/H

21. *Dryopteridaceae* Herter

Arachniodes Blume

^E *Arachniodes insularis* W.H.Wagner

Distribution: K/O/Mo/Ma/H

Ctenitis (C. Chr.) C. Chr.

^E *Ctenitis latifrons* (Brack.) Copel.

Distribution: K/O/Mo/L/Ma/H

^E *Ctenitis squamigera* (Hook. & Arn.) Copel.

Distribution: K/O/Mo/L/Ma

USFWS category: Endangered

Cyrtomium C. Presl

- ^I ***Cyrtomium caryotideum*** (Wall. ex Hook. & Grev.) C.Presl
Distribution: K/O/Mo/L/Ma/H
- ^{NZ} ***Cyrtomium falcatum*** (L.f.) C.Presl
Distribution: K/O/Mo/L/Ma/H
- Dryopteris*** Adans.
- ^E ***Dryopteris crinalis*** (Hook. & Arn.) C.Chr.
var. ***crinalis***
Distribution: K/O/Mo/L/Ma/H
var. ***podosora*** (W.H.Wagner) D.D.Palmer
Distribution: K
USFWS category: Endangered
- ^E ***Dryopteris fuscoatra*** (Hillebr.) W.J.Rob.
var. ***fuscoatra***
Distribution: K/O/Mo/L/Ma/H
var. ***lamoureuxii*** Fraser-Jenk.
Distribution: Ma
- ^E ***Dryopteris glabra*** (Brack.) Kuntze
var. ***alboviridis*** (W.H.Wagner) D.D.Palmer
Distribution: K
var. ***flynnii*** D.D.Palmer
Distribution: K
var. ***glabra***
Distribution: K/O/Mo/L/Ma/H
var. ***hobdyana*** (W.H.Wagner) D.D.Palmer
Distribution: Ma
var. ***nuda*** (Underw.) Fraser-Jenk.
Distribution: K/O/Mo/Ma
var. ***pusilla*** (Hillebr.) Fraser-Jenk.
Distribution: K
USFWS category: Endangered
var. ***soripes*** (Hillebr.) Herat ex Fraser-Jenk.
Distribution: K/O/Mo/Ma/H
- ^E ***Dryopteris hawaiiensis*** (Hillebr.) W.J.Rob.
Distribution: K/O/Mo/Ma/H
- ^E ***Dryopteris mauiensis*** C. Chr.
Distribution: K/O/Mo/L/Ma/H
- ^E ***Dryopteris rubiginosa*** (Brack.) Kuntze
Syn. *Nothoperanema rubiginosum* (Brack.) A.R.Sm. & D.D.Palmer
Distribution: K/O/Mo/L/Ma/H
- ^E ***Dryopteris sandwicensis*** (Hook. & Arn.) C.Chr.
Distribution: K/O/Mo/L/Ma/H
- ^E ***Dryopteris subbipinnata*** W.H.Wagner & Hobdy
Distribution: Ma/H
- ^E ***Dryopteris tetrapinnata*** W.H.Wagner & Hobdy
Distribution: Ma
- ^E ***Dryopteris unidentata*** (Hook. & Arn.) C.Chr.

var. **paleacea** (Hillebr.) Herat ex Fraser-Jenk.

Distribution: K/O/Mo/Ma/H

var. **unidentata**

Distribution: K/O/Mo/L/Ma/H

^I **Dryopteris wallichiana** (Spreng.) Hyl.

Distribution: K/O/Mo/Ma/H

Elaphoglossum Schott ex J.Sm.

^E **Elaphoglossum aemulum** (Kaulf.) Brack.

Distribution: K/O/Mo/L/Ma/H(ex)

^E **Elaphoglossum alatum** Gaudich.

Distribution: O

^E **Elaphoglossum crassicaule** Copel.

Distribution: K

^E **Elaphoglossum crassifolium** (Gaudich.) W.R.Anderson & Crosby

Distribution: K/O/Mo/L/Ma/H

^E **Elaphoglossum fauriei** Copel.

Distribution: O/Mo

^I **Elaphoglossum paleaceum** (Hook. & Grev.) Sledge

Distribution: K/O/Mo/L/Ma/H

^E **Elaphoglossum parvisquameum** Skottsbo.

Distribution: Mo/L/Ma/H

^E **Elaphoglossum pellucidum** Gaudich.

Distribution: K/O/Mo/Ma/H

^E **Elaphoglossum wawrae** (Luer ss.) C.Chr.

Distribution: K/O/Mo/Ma/H

^E **Elaphoglossum alatum** Gaudich. × **E. fauriei** Copel.

Distribution: O

^E **Elaphoglossum fauriei** Copel. × **E. parvisquameum** Skottsbo.

Distribution: Mo

Polystichum Roth

^E **Polystichum bonseyi** W.H.Wagner & Hobdy

Distribution: Ma/H

^E **Polystichum haleakalense** Brack.

Distribution: Ma/H

^E **Polystichum hillebrandii** Carruth.

Distribution: Ma/H

22. **Nephrolepidaceae** Pic.Serm.

Nephrolepis Schott

^{NZ} **Nephrolepis brownii** (Desv.) Hovenkamp & Miyam.

Syn. **Nephrolepis multiflora** (Roxb.) F.M.Jarrett ex C.V.Morton

Distribution: N/K/O/Mo/L/Ka/Ma/H

^I **Nephrolepis cordifolia** (L.) C.Presl

Distribution: K/O/Mo/L/Ma/H

Nephrolepis exaltata (L.) Schott

^E subsp. **hawaiiensis** W.H.Wagner

Distribution: N/K/O/Mo/L/Ma/H

^{NZ} ***Nephrolepis falcata*** (Cav.) C.Chr. ‘**Furcans**’

Distribution: K/O/Mo/L/Ma/H

^{NZ} ***Nephrolepis hirsutula*** (G. Forst.) C.Presl ‘**Superba**’

Distribution: K/Ma

^E ***Nephrolepis* × *copelandii*** W.H.Wagner (*Nephrolepis cordifolia* × *N. brownii*)

Distribution: O

^E ***Nephrolepis* × *medlerae*** W.H.Wagner (*Nephrolepis exaltata* subsp. *hawaiiensis* × *N. brownii*)

Distribution: O

23. **Tectariaceae** Panigrahi

Tectaria Cav.

^E ***Tectaria gaudichaudii*** (Mett.) Maxon

Distribution: K/O/Mo/L/Ma/H

^{NZ} ***Tectaria incisa*** Cav.

Distribution: K/O/Ma/H

24. **Davalliaceae** M.R.Schomb. ex A.B.Frank

Davallia Sm.

^{NZ} ***Davallia fejeensis*** Hook.

Distribution: O

25. **Polypodiaceae** Bercht. & J.Presl

Adenophorus Gaudich.

^E ***Adenophorus abietinus*** (D. C. Eaton) K.A.Wilson

Distribution: K/O/L/Ma

^E ***Adenophorus epigaeus*** (L.E.Bishop) W.H.Wagner

Distribution: K

^E ***Adenophorus haalilioanus*** (Brack.) K.A.Wilson

Distribution: K/O

^E ***Adenophorus hymenophylloides*** (Kaulf.) Hook. & Grev.

Distribution: K/O/Mo/L/Ma/H

^E ***Adenophorus oahuensis*** (Copel.) L.E.Bishop

Distribution: O

^E ***Adenophorus periens*** L.E.Bishop

Distribution: K/O(ex)/Mo/L(ex)/Ma(ex)/H

USFWS category: Endangered

Note: Extant population status on K, Mo, and H needs verification - possibly extinct

^E ***Adenophorus pinnatifidus*** Gaudich.

var. ***pinnatifidus***

Distribution: K/O/Mo/L/Ma/H

var. ***rockii*** (Copel.) D.D.Palmer

Distribution: K/O/Mo/Ma

^E ***Adenophorus tamariscinus*** (Kaulf.) Hook. & Grev.

var. ***montanus*** (Hillebr.) L.E.Bishop

Distribution: Mo/Ma/H

var. ***tamariscinus***

Distribution: K/O/Mo/L/Ma/H

^E ***Adenophorus tenellus*** (Kaulf.) Ranker

Syn. *Grammitis tenella* Kaulf.

Distribution: K/O/Mo/L/Ma/H

^E ***Adenophorus tripinnatifidus*** Gaudich.

Distribution: K/O/Mo/L/Ma/H

^E ***Adenophorus* × *abbottiae*** W.H.Wagner (*Adenophorus hymenophylloides* × *A. tamariscinus*)

Distribution: K/O

^E ***Adenophorus* × *carsonii*** Ranker (*Adenophorus hymenophylloides* × *A. tripinnatifidus*)

Distribution: H

^E ***Adenophorus oahuensis*** (Copel.) L.E.Bishop × ***A. pinnatifidus*** Gaudich.

Syn. *Oligadenus* × *bishopii* W.H.Wagner

Distribution: O

Aglaomorpha Schott.

^{NZ} ***Aglaomorpha rigidula*** (Sw.) Hovenkamp & S.Linds.

Syn. *Drynaria rigidula* (Sw.) Bedd.

Distribution: O

New record (Lau and Frohlich, 2013; reported as *D. rigidula*)

Lepisorus (J.Sm.) Ching

[†] ***Lepisorus thunbergianus*** (Kaulf.) Ching

Distribution: K/O/Mo/L/Ma/H

Microsorium Link

^{NZ} ***Microsorium grossum*** (Langsd. & Fisch.) S.B.Andrews

Syn. *Phymatosorus grossus* (Langsd. & Fisch.) Brownlie

Distribution: K/O/Mo/L/Ma/H

Note: *M. grossum* and *M. scolopendria* may be conspecific; see Nooteboom (1997) and Krier et al. (2008)

^{NZ} ***Microsorium scolopendria*** (Burm.f.) Copel.

Syn. *Phymatosorus scolopendria* (Burm.f.) Pic.Serm.

Distribution: Ma

M. grossum and *M. scolopendria* may be conspecific; see Nooteboom (1997) and Krier et al. (2008)

^E ***Microsorium spectrum*** (Kaulf.) Copel.

var. ***pentadactylum*** (Hillebr.) D.D.Palmer

Distribution: K/Ma

var. ***spectrum***

Distribution: K/O/Mo/L/Ma/H

Oreogrammitis Copel.

^E ***Oreogrammitis baldwinii*** (Baker) Parris

Syn. *Grammitis baldwinii* (Baker) Copel.

Distribution: K

^E ***Oreogrammitis forbesiana*** (W.H.Wagner) Parris

Syn. *Grammitis forbesiana* W.H.Wagner

- Distribution: K/Mo/Ma
^E ***Oreogrammitis hookeri*** (Brack.) Parris
 Syn. *Grammitis hookeri* (Brack.) Copel.
 Distribution: K/O/Mo/L/Ma/H
- Phlebodium*** (R.Br.) J.Sm.
^{NZ} ***Phlebodium aureum*** (L.) J.Sm.
 Distribution: K/O/Mo/L/Ma/H
- Platynerium*** Desv.
^{NZ} ***Platynerium bifurcatum*** (Cav.) C.Ch.
 Distribution: O/Ma/H
^{NZ} ***Platynerium superbum*** de Jonch. & Hennipman
 Distribution: O
- Polypodium*** L.
^E ***Polypodium pellucidum*** Kaulf.
 var. ***acuminatum*** D.D.Palmer
 Distribution: K/Ma
 var. ***pellucidum***
 Distribution: K/O/Mo/L/Ma/H
 Includes: *P. pellucidum* Kaulf. var. *pellucidum* f. *pellucidum* and *P. pellucidum* Kaulf. var. *pellucidum* f. *opacum* (Hillebr.) D.D.Palmer
 var. ***vulcanicum*** Skottsb.
 Distribution: Mo/Ma/H
- Pyrrosia*** Mirb.
^{NZ} ***Pyrrosia longifolia*** (Burm.f.) C.V.Morton
 Distribution: O
 New record (Frohlich and Lau, 2014)
^{NZ} ***Pyrrosia piloselloides*** (L.) M.G.Price
 Distribution: O
 New record (Lau and Frohlich, 2012)
- Stenogrammitis*** Labiak
^E ***Stenogrammitis saffordii*** (Maxon) Labiak
 Syn. *Lellingeria saffordii* (Maxon) A.R.Sm. & R.C.Moran
 Distribution: K/O/Mo/L/Ma/H

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SHORTER NOTE

Occasional Bifid Leaves in *Selaginella helvetica*.—The lycophyte leaf is unique having evolved independently of the leaves of euphyllophytes (Tomescu, Trends in Plant Science 14:5–12. 2008). The leaves of lycophytes are commonly referred to as microphylls and first formed as enations of the stem. Into this enation, a single vascular trace extending from the stele infiltrated to facilitate hydration and the transport of photosynthates (Stewart and Rothwell, *Paleobotany and the evolution of plants Second Edition*, Cambridge University Press, Cambridge. 1993). Unlike the leaves of euphyllophytes, the unique developmental origin of lycophyte microphylls is believed to have placed considerable developmental constraint on the evolution of compound leaves and reticulate venation in this lineage (Harrison, Rezvani and Lugdale, Development 134:881–889. 2007). With only a few exceptions, microphylls in extant species have a single vascular trace in a simple, unlobed lamina. Reported variants to this single-vein vascular arrangement in microphylls include an occasional two or more veins in the simple leaves (or leaves with small lobes at the apex) of the extant species *Selaginella willdenowii* (Desv.) Baker (Grambast and Rosello, Comptes rendus de l'Académie des Sciences 261: 5183–5186. 1965) and *S. martensii* Spring (Webster, American Fern Journal 60:1–6. 1970). While the most unusual extant exception within the lycophyte clade is the branching veins in the simple leaves of the species *S. schaffneri* Hieron. (an example of this unique vascular arrangement is shown in Fig. 1A) and *S. adunca* A.Braun ex Hieron. (Wagner, Beitel, Wagner, Science 218:793–794. 1982). A new and undescribed variant to the simple and single-veined microphyll leaves of lycophytes can be found on occasion in *S. helvetica* (L.) Spring (Fig. 1C and 1D). During work involving the close observation of stems and leaves of an individual of this species, collected in Southern Germany and subsequently cultivated in the greenhouses of Purdue University, Indiana, a number of bifid leaves were observed across numerous shoots. Two unique forms were observed. The first form found in the larger ventral leaves, had two equal sized lobes each containing a vein originating from a common vascular trace. These veins bifurcate at the base of the leaf and extend to the apex of each of the lobes (Fig. 1). The second form, observed in smaller dorsal leaves, had a short single vein extending to the terminus of the sinus separating two uneven sized lobes (Fig. 1). In both bifid leaf forms, a single and entire ligule was present (Fig. 1). Bifid leaves are not known in any extant lycophyte species; however, these variants in *S. helvetica* indicate that the genetic or developmental capacity for such an innovation exists in this lineage. Branched or lobed leaves in lycophytes have been described in a number of extinct genera and species including *Estinnophyton*, *Protolepidodendron*, *Spencerites insignis* and the genus *Leclercqia* (Stewart and Rothwell, 1993). *Leclercqia* species have the most comprehensively described and well-preserved leaves of these extinct, lobe-leaved lycophyte

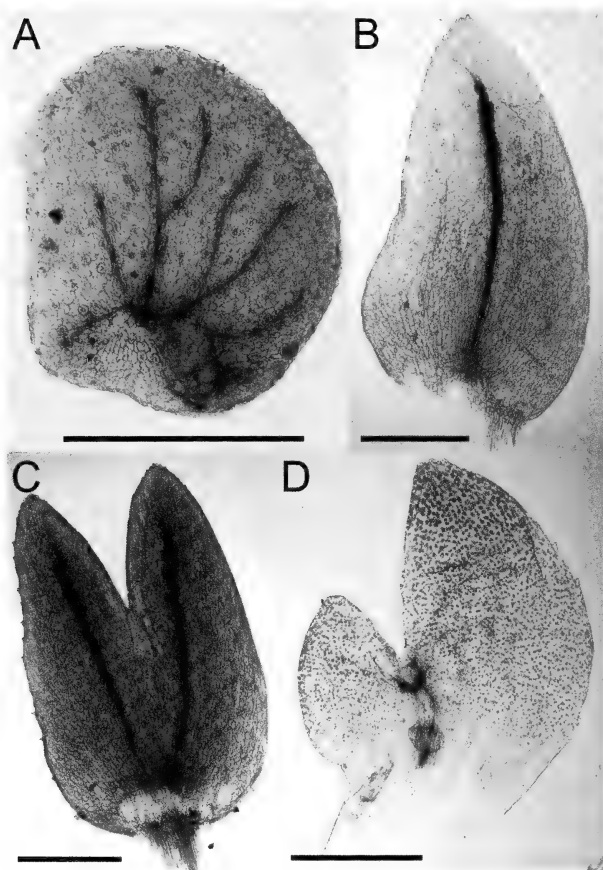


FIG. 1. A. The simple leaf of *Selaginella schaffneri* displaying a unique branching and dichotomising venation arrangement (from herbarium specimen PH00023406). B. A common simple leaf of *S. helvetica*. C. A bifid ventral leaf of *S. helvetica* with veins extending to the apex of the leaf. D. A bifid dorsal leaf of *S. helvetica* with vein extending to the terminus of the sinus of the two lobes. All leaves were cleared with dilute sodium hypochlorite solution and stained with Toluidine Blue O, the leaves of *S. helvetica* were taken from the same plant, voucher accession PUL N18230. Bars = 500 μm .

species; the leaves of *Leclercqia* species are divided at the tip into five, and up to seven, lobes (Bonamo, Banks and Grierson, Botanical Gazette 149:222 – 239. 1988). In some *Leclercqia* species, lateral veins may have branched from a central main vascular trace to supply each of these lobes (Bonamo and Grierson, Botanical Society of America Miscellaneous Series 160:42. 1981). Other extinct leaf shape or venation variants in lycophytes include the two-veined leaves of *Sigillaria* (Stewart and Rothwell, 1993), and the sagittate, not lanceolate, leaves of *Haskinsia* (Bonamo, Banks and Grierson, 1988). These examples suggest that a complex leaf form and venation arrangement is developmentally possible in the lycophyte lineage, and has been for much of its history. The foliar and venation variants so far described in lycophytes

mirror (at a smaller scale) much of the anatomical variation of euphyllophyte leaves. This raises the important question of why, given the developmental capacity, has this variation not been acted upon by natural selection? This is especially perplexing considering the physiological benefits of increased photosynthetic capacity conferred by reticulate venation (Brodribb, Feild and Jordan, *Plant Physiology* 144:1890–1898. 2007). A potential explanation for this lack of adoption of euphyllophyte leaf modification broadly across the lycophyte lineage remains unknown.—SCOTT A. M. McADAM, Purdue University, Department of Botany and Plant Pathology, Purdue Center for Plant Biology, 915 W. State St, West Lafayette, IN, 47907, USA, email: smcadam@purdue.edu.

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COVER CAPTION: The simple leaf of *Selaginella schaffneri* displaying a unique branching and dichotomising venation arrangement. Photo credit: Scott McAdam